1-1-2003

Flavor characteristics of irradiated apple cider during storage: effects of packaging conditions and sorbate addition

Loretta Rose Crook
Iowa State University

Flavor characteristics of irradiated apple cider during storage: Effects of packaging conditions and sorbate addition

by

Loretta Rose Crook

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Terri D. Boylston, Major Professor
Cheryll A. Reitmeier
Lester A. Wilson
Gail Nonnecke

Iowa State University

Ames, Iowa

2003

Graduate College
Iowa State University

This is to certify that the master's thesis of

Loretta Rose Crook

has met the thesis requirements of Iowa State University

TABLE OF CONTENTS

ABSTRACT

The United States Food and Drug Administration requires a 5-$\log_{10}$ reduction in populations of the pathogen *E. coli* 0157:H7 in order to sell apple cider without a warning label. Heat pasteurization, the current process used to reduce microbial load and deem apple cider safe to consume, can cause undesirable changes to color, flavor and viscosity attributes. Electron beam irradiation, therefore, has been investigated as a non-thermal processing method to inactivate food-borne pathogens and reduce spoilage of fresh apple cider.

Flavor quality changes which occur during long-term storage of apple cider can be monitored by attributes such as analytical measurements (soluble solids, pH, acidity) and volatile flavor profiles. The objective of this research was to evaluate the flavor quality of irradiated apple cider. Experiment I studied the effects of sorbate addition and three packaging materials (polystyrene, nylon-6 and low-density polyethylene) to maintain characteristic apple cider flavor during three weeks of refrigerated storage. Experiment II evaluated the effects of sorbate addition and three gaseous environments (atmospheric air, oxygen-flush and nitrogen-flush) to maintain characteristic apple cider flavor during seven weeks of refrigerated storage. The control sample in both experiments was unirradiated apple cider.

Quality attributes displayed general trends as a result of fermentation and other degradation reactions which take place in apple cider throughout storage. Typically, as a result of sugar conversion by yeasts into acids and alcohols, soluble solids decreased while available acids increased with time. Volatile flavor compounds were evaluated by plotting rates of change using gas-chromatography peak areas versus time. The rates of change were determined on a logarithmic scale to quantify the effects of storage.

Based on the results of Experiment I, cider irradiated and stored in polystyrene and nylon-6 packaging materials had lower rates of volatile flavor loss than unirradiated cider and irradiated cider packaged in low-density polyethylene. Data shown in Experiment II suggested that nitrogen-flush and atmospheric air conditions were better able to maintain characteristic apple cider flavors during storage than an oxygen-flush environment. Potassium sorbate was very effective in extending the shelf-life of apple cider. The rates of loss during storage for a majority of the volatile flavor compounds were significantly lower in the presence of 0.1% sorbate than in the absence of sorbate. Analytical measurements also supported the effect of sorbate to minimize changes in soluble solids and acidity during refrigerated storage.

INTRODUCTION

Apple Industry

Apples (*Malus pulima* Mill.) are one of the most popular fruits in the world. In the United States, apples accounted for approximately 13% of all fruit produced between 1996 and 2000 (USDA, 2003). About 30,000 apple growers in thirty-five states produce apples commercially. A majority of this commodity is consumed as whole apples, apple juice, or apple cider. Apple cider is a minimally processed form of juice from whole apples. Although there is no single, legal definition for apple cider, a common description considers it as "the juice from freshly squeezed apples separate from pomace with no further clarification" (Kozempel et al., 1998). The production of apple cider is a wonderful utilization of apple surpluses or apples that are not considered of the highest quality for fresh apple consumption.

Apples, like most fruits, consist of a complex blend of organic flavor compounds which create a unique and characteristic flavor profile. The combinations of such flavor compounds can vary greatly with apple cultivar, state of maturity and other factors. In addition, the process of crushing apples to produce apple cider can create unique flavor profiles. The naturally acidic environment of apple cider provides some defense against microorganisms, but manufacturing and processing conditions used by a producer can ultimately influence the quality and safety of apple cider.

Health Concerns

The quality and safety of fresh apple cider is of great concern for the public and governmental regulatory agencies. Many microorganisms can cause foodborne illnesses in unfermented, unpasteurized apple cider. Young children, the elderly and immune system-

compromised individuals are especially susceptible to such foodborne illness. *Salmonella typhimurium*, *Cryptosporidium parvum*, and most commonly *Escherichia coli* 0157:H7 have been responsible for a majority of the foodborne illness outbreaks linked to apple cider (Ingham and Schoeller, 2002). These organisms are resistant to low pH (3.3-4.1) and low storage temperatures (Jay, 2000). Human foodborne illness, as a result of *E. coli* contamination in apple cider, may occur through many possible modes of contamination, such as contact with bacteria in fecal material from the soil, improper handling of fruit or unsanitary manufacturing processes (Kozempel et al., 1998). As a result of such outbreaks, the United States Food and Drug Administration (FDA) mandated a 5-$\log_{10}$ reduction in the organism of interest that could be present in apple cider, specifically *E. coli* 0157:H7. If this 5-$\log_{10}$ reduction is not achieved, a special warning label must be present on apple cider in order to be sold for human consumption (FDA, 1998). This rule has been in effect since September 8, 1998.

Importance of Study

Currently, pasteurization is the processing step applied by cider producers or manufacturers to obtain a 5-$\log_{10}$ reduction in target pathogens. Such practices also extend shelf-life by reducing spoilage organisms. The high temperatures cider is exposed to during pasteurization can have a negative or undesirable impact on characteristic flavor attributes. Processes such as the addition of preservatives or electron beam irradiation, however, are being investigated as alternative methods to extend shelf life yet retain typical apple cider flavors. The addition of sorbate increases the shelf-life of apple cider by controlling the growth of yeasts and molds.

This study was conducted to investigate how packaging treatment and the gas environment which apple cider is exposed to during irradiation and storage can contribute to the retention of characteristic apple cider flavors. The first experiment evaluated the effects of three packaging materials (polystyrene, nylon-6 film and low density polyethylene film) used during the irradiation and storage of apple cider compared to a control, unirradiated samples stored in glass jars. In the second experiment, cider to be irradiated was exposed to three gaseous environments (oxygen-flush, nitrogen-flush and atmospheric air) and compared to unirradiated samples exposed to atmospheric air. Volatile flavor analysis using solid-phase microextraction techniques and analytical measurements such as soluble solids, pH and titratable acidity were used to examine the retention of characteristic apple cider flavor throughout up to seven weeks of refrigerated storage following irradiation treatment.

LITERATURE REVIEW

Composition of Apples

Apples and apple products have characteristic taste and flavor attributes which differ from other fruits. In addition, each specific apple cultivar can have a unique blend of organoleptic and quality traits. Production regions, seasonal variation and apple maturity also impacts the composition of apples and apple products.

Flavor compounds

Over the last few decades, a large amount of research has been dedicated to the identification of flavor compounds in apples and apple cider. Various publications have explored the composition of esters, alcohols, carbonyls, acetals, halogenated compounds and even lactones and hydrocarbons in apple products (Flath et al., 1967; Dimick and Hoskin, 1983; Williams et al., 1981). Dimick and Hoskin (1983) provided a list of 266 compounds which have been isolated from apples and apple products. A challenge exists in correlating chemical compounds with sensory responses.

The contribution of specific flavor compounds to sensory characteristics has been investigated. A synthetic apple juice odor was developed by Durr and Rothlin (1981) based on sensory testing and volatile flavor analyses of characteristic apple essences (Flath et al., 1967; 1969). The synthetic flavor formula that most likely resembled that of natural apple juice essence contained trans-2-hexenal, ethyl butanoate, ethyl 2-methylbutanoate, pentyl acetate, 1-butanol, 2-methylbutanol, 1-hexanol, hexanal, and benzaldehyde. The contribution of each compound to a specific odor is complex. A few of the descriptions used to describe apple odor are apple, fruity, pomace, sweet, grassy, floral, cooked apple, almond, alcoholic and solvent (Durr and Rothlin, 1981).

Many studies have been completed to compare sensory attribute changes to individual volatile compounds. Studies by Dimick and Hoskin (1983) and Young et al. (1996) identified the volatile flavor compounds which contribute to characteristic apple flavor and aroma. Increasing levels of hexyl acetate, 2-methylbutyl acetate and/or butanol were correlated to an increase in sensory "overall flavor and aroma", "sweet aroma", and/or more specifically "apple aroma and flavor". A study by Lopez et al. (1998) based the intensity of volatile compounds compared to characteristic aroma and flavor properties of apples. A "sweetish sensation" was attributed to 1-hexanol and 1-butanol while "sweet-fruity" flavor was attributed to hexyl acetate. More specifically, apple aroma was characterized by the presence of ethyl propionate and butyl acetate (Lopez et al., 1998).

The combination of acids and sugars are two major elements which also influence the taste of a food product. Apples and apple cider contain a mixture of sugars (especially fructose, glucose, sucrose, and sorbitol), oligosaccharides and polysaccharides (i.e. starch), together with organic acids (malic, quinic, shikimic) and amino acids (aspartate, glutamate, asparagine, serine, glutamine, leucine, glycine, arginine, alanine, valine, phenylalanine) (Dauthy, 1995). A sugar/acid ratio is very often used to determine the balance of sweetness and tartness in fruit and vegetable products. A sugar/acid ratio is often used to group apple cultivars according to primary flavor characteristics (Appendix A.2). Soluble solids and titratable acidity levels influence the acceptability of a food product and relate to overall sweetness and tartness attributes.

Phenolic compounds

Fruit juices are known to contain many health-beneficial compounds and juice from apples contains a high level of natural phenolic compounds. Hydroxycinnamic acid

derivatives, flavan-3-ols, dihydrochalcones or flavonols are phenolic compounds that can serve as natural antioxidants by scavenging free radicals. Total phenol content and vitamin C concentration of fruit juices is strongly correlated to antioxidant capacity (Gardner et al., 2000). Phenolic antioxidants present in fruit juices also protect vitamin C (ascorbic acid) from oxidative degradation (Miller and Rose-Evans, 1997).

Polyphenol content has been correlated with a distinguishable bittersweet (Lea and Timberlake, 1978) or bitter-astringent (Poll, 1981) characteristic of apples. The study by Poll (1981) reported a direct correlation between polyphenol content and bitter-astringent intensity for eighteen apple cultivars. The brown color of cider is essentially caused by phenolic compounds which are involved in oxidation reactions (Sanoner et al., 1999). However, brown color is also dependent on enzyme (primarily polyphenol oxidase) and vitamin C concentrations (Poll, 1981). Phenol content, therefore, is another important factor which affects the quality parameters of apple cider and varies according to apple cultivar, apple maturity and growing conditions.

Factors Affecting the Quality and Flavor of Apples and Apple Cider

Apple quality, especially the concentration of aroma compounds, changes with degree of ripeness. In addition to cultivar type, variation in apple flavor can be caused by additional factors. Internal metabolic ripening such as genetic alterations, ethylene and respiration rates can vary by season or year. External factors such as fruit cultivation, i.e. climate, soil and fertilization, can also be responsible for flavor variations (Lopez et al., 1998).

Apple classification

Categorization of cider apples into four major classifications expresses the variation among apple cultivars. Traditional English classification categorizes cider apples into four

types: bittersweets, bittersharps, sweets, and sharps. "Bitter" is associated with high polyphenol content, "sharp" is associated with high acid content, and "sweet" is associated with low acid content (Dimick and Hoskin, 1983). Apple cultivars with a high sugar:acid ratio are considered to be "very sweet" and those with low sugar:acid ratios were designated as "very sour" (Poll, 1981).

Each apple cultivar has a unique profile of volatile flavor compounds. Williams et al. (1981) compared the volatile flavor compounds of four cider apple cultivars, Sweet Coppin (sweet), Bramley's Seedling (sharp), Kingston Black (bittersweet), and Yarlington Mill (bittersharp). Overall, Kingston Black and Yarlington Mill cultivars had a higher concentration of aroma components, but Bramley's Seedling and Yarlington Mill cultivars had more characteristic esters. More specifically, acetate esters dominated among all four cultivars, but relative amounts of other esters vary with each cultivar (Williams et al., 1981).

Lopez et al. (1998) compared the volatile compounds of two apple cultivars, Golden Delicious and Granny Smith, using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. Significant differences were reported in total volatiles between two seasons of harvest, 1993 and 1994, which could be attributed to factors, including apple maturity. In the 1994 season, 79% of total volatiles in 'Golden Delicious' apples were attributed to ethyl acetate, ethyl propionate, propyl acetate, butyl acetate, 2-methylpropyl acetate and ethyl 2-methyl butanoate. In the same season, 76% of total volatiles found in 'Granny Smith' apples were attributed to ethyl propionate, propyl acetate, butyl acetate, 2-methyl propyl acetate, ethyl acetate, pentyl acetate and t-butyl propionate (Lopez et al., 1998). Similar volatile flavor profiles exist between the two apple cultivars, but the amount of each compound varies. In a separate study by Young et al. (1996), 2-

methylbutyl acetate, butyl acetate, hexyl acetate, and butanol were the most prevalent flavor compounds reported in 'Royal Gala' apples. As mentioned above, the amounts or presence of characteristic flavor compounds varies for each apple cultivar.

Ripening/maturity factors

Ethylene exposure is known to accelerate certain responses in fruit development. Stimulation of respiration rates and lipoxygenase activity, therefore, encourages the ripening process. Song and Bangerth (1994) evaluated the effects of butanal (aldehyde) and ethylene treatment on the formation of aroma compounds in 'Golden Delicious' apples. This study found that esters and alcohols, especially butyl acetate, hexyl acetate, butanol, and 2-methylbutyl acetate, are dominant in fully matured apples. Following butanal treatment, the conversion of this aldehyde into alcohols and corresponding aromatic esters takes place at a faster rate (Song and Bangerth, 1994). During the natural ripening processes, however, lipoxygenase is responsible for the production of aldehydes from the β-oxidation of fatty acids such as palmitic, stearic, oleic, and linoleic acid. Since the fatty acid content is also dependant on apple maturity, this is a major limiting factor for aroma production in immature fruits. Overall, aroma production increases progressively with an increase in fruit maturity. For this reason, cider made from immature apples will not be as aromatic or flavorful as cider made from fully developed apples.

Traditionally, starch content and a total sugars/total acidity ratio have been used to evaluate the optimum ripening stage of cider apples. Plestenjak et al. (1994), evaluated several parameters to calculate a maturity index. Five apple cultivars were harvested at one-week harvest intervals for five weeks and were evaluated at harvest and after a period of six months in controlled atmosphere (CA= 1°C, 1.5% O_2, and 1.5% CO_2) storage conditions.

Firmness (F) was measured using a hand penetrometer, which measures the depth of penetration using a cone of set weight, diameter and angle during a specific period of time. Starch content (R) was determined by coloring half an apple with potassium-iodine solution and using visual comparison of starch disappearance with a standard 10-step template. A higher starch index corresponds to less starch and more conversion into sugar (i.e. more mature fruit). Some studies propose a scale of five instead of ten for starch index (Travers et al., 2002). Soluble solids (S) were determined by Plestenjak et al. (1994) using a hand refractometer which bases light refraction changes on sugar content. Titratable acids or available acids were measured by means of titration with sodium hydroxide. Based on these parameters, a maturity index value was estimated as F/RS. In terms of harvest time, F/RS decreased with a later harvest date since firmness decreases and both starch index values and soluble solids content increases with maturation on the tree (Plestenjak et al., 1994).

Quantification of ripeness and optimum harvest time, based on several chemical and biochemical processes, is quite complicated and cannot be based on selected compounds or apple cultivar classification. In the final stages of apple ripening, a decrease in starch, organic acids and certain amino acids occurs while an increase in sugars and soluble pectins takes place (Mangas et al., 1998). Amino acids present in apples can also serve as precursors of characteristic aroma compounds such as higher alcohols in ripening or cider processing.

Storage conditions

Storage conditions of whole apples can affect apple flavor composition, even though the peel creates an oxygen barrier. The study by Plestenjak et al. (1994), which created a maturity index for apples, found that controlled atmosphere storage (CA=1.5% O_2, 1.5%

CO_2, 1°C) increased soluble solids and decreased firmness and titratable acidity. The rates of such changes varied with each apple cultivar but the effects of storage follow this trend.

According to a study by Boylston et al. (1994), firmness of 'Gala' apples decreases significantly following 3-4 months of storage. Softening during storage can be attributed to changes in the pectin fractions. Pectin fractions of apples vary according to solubility characteristics. According to Mangas et al. (1992), the proportion of water soluble pectins increases during the final ripening stages while those that are water-insoluble decrease.

Possible microbial concerns of stored apples can also be limited under certain storage environments. Conditions such as cleanliness, temperature and gas environment conditions can greatly impact the quality of a final product. Clean apples, water loss and oxygen prevalence decreases the opportunity for anaerobic metabolism to encourage excessive ethanol production by means of alcoholic fermentation (Dimick and Hoskin, 1983).

Temperature

Respiration rates, which encourage ripening or decay processes, increase with temperature. In order to promote desirable ripening but prevent decay, an appropriate storage temperature should be maintained. Storing apples at 0-4°C will slow respiration rates and inhibit growth of decay organisms, but this temperature may also reduce ester formation. Enzymatic systems required for ester formation are dependent on storage temperature.

Based on findings reported by Dimick and Hoskin (1983), 'Red Delicious' apples stored at 1°C yielded volatile components at a maximum concentration at 2-4 months, which demonstrates climacteric ripening. When apples were stored at room temperature, practically no esters were produced in the first four weeks of storage. After four weeks, however, ester production did occur, along with undesirable wilting and textural changes. Temperature of

ripening affected volatile output; 46°C inhibited aroma production, 32°C increased the rate of ester production and 22°C maximized total ester yields (Dimick and Hoskin, 1983). According to these findings, apple quality, based on textural and flavor attributes, will be better when apples are stored at 1°C compared to room temperature.

Another study by Wills and McGlasson (1971) evaluated the effects of storage temperature on weight loss. 'Jonathan' apples were stored at five temperatures between -1°C to 10°C (-1°, 0°, 2.5°, 5°, and 10°C) and weight loss was calculated after 1, 2, 3, 4, 6, 9 and 12 weeks in storage. The study found that the rate of weight loss increased as storage temperature increased because rates of evaporation, dehydration, respiration and diffusion are higher. This is quite pertinent to the storage effects of apples used for cider processing since weight loss can decrease juice yield, making the process less efficient. In addition to lower temperatures, a high humidity in the storage environment of whole apples will also reduce moisture loss.

Oxygen concentration

Controlled atmosphere (CA) storage is a technique which was developed to hold fruit and vegetables in sealed environments under controlled temperature and gas (oxygen, nitrogen and carbon dioxide) conditions. Compared to regular atmosphere (RA) storage, CA storage (low O_2 and high CO_2) extends shelf-life by reducing fruit respiration, delaying ripening and minimizing losses due to shriveling and other physiological disorders. Volatile production, however, can be negatively affected as a result of CA storage (Plotto et al., 1999).

Boylston et al. (1994) studied the effects of storage at regular atmosphere (RA at 0°C) compared to controlled atmosphere (CA = 1% O_2, 1% CO_2, 0°C) conditions on the volatile

flavor profiles of 'Gala' apples. The content of specific volatile compounds, such as ethyl butanoate, butyl hexanoate, hexyl hexanoate, t-2-hexenal, octanal and nonanal, were significantly higher in RA than in CA apples. The difference is attributed to low-oxygen environments present in CA storage. Overall, during four months of storage, natural senescence was thought to contribute to the significant loss of characteristic compounds such as butyl and hexyl acetate in both RA and CA conditions. Differences in volatile flavor compounds were also found between apples from three different orchards which were evaluated in the study. The lot which had the higher sensory acceptance ratings had higher soluble solids and titratable acidity as well as higher contents of several characteristic volatile flavor compounds known to be present in apples (Boylston et al., 1994).

Anaerobic storage conditions can also bring about high amounts of certain volatile compounds compared to oxygen-rich environments. Compared to control apples (stored at 1.5% O_2 and 2.0% CO_2), whole 'Delicious' apples stored for 30 days under anaerobic storage conditions (0.05% O_2 and 0.2% CO_2) developed large concentrations of ethanol and acetaldehyde. Subsequent increases in ethyl esters, especially ethyl acetate, tended to displace the ordinarily predominant butyl esters, hexyl esters, aldehydes and ketones (Mattheis et al., 1991). Increased ethanol production modifies the flavor profile of apples stored in an anaerobic environment compared to those stored in an aerobic environment. Further conversion into ethyl esters may encourage volatilization and loss of such esters into the surrounding environment. When an adequate supply of oxygen is not present in a storage environment, anaerobic metabolism replaces aerobic metabolism with a concurrent increase in alcohol (Dimick and Hoskin, 1983).

Apple Cider Production

The manufacturing steps used by each apple cider producer can vary. In general, whole apples are inspected and/or washed to remove debris and apples which do not meet satisfactory quality. Next, apples are chopped into a pomace (using a grater, chopper, mill, etc.) to a particle size between ¼ to ¾ inches and accumulated into a pressing bin. Juice is extracted from the pomace by a force of compression and passes through some type of screening to remove large particles of skin, flesh and seeds that remain. Further processing treatments, such as the addition of preservatives and/or pasteurization/irradiation/ultraviolet light, can be completed prior to bottling and distribution. An ideal system of operations for efficiently producing safe, high-quality apple cider is provided in Figure 1.

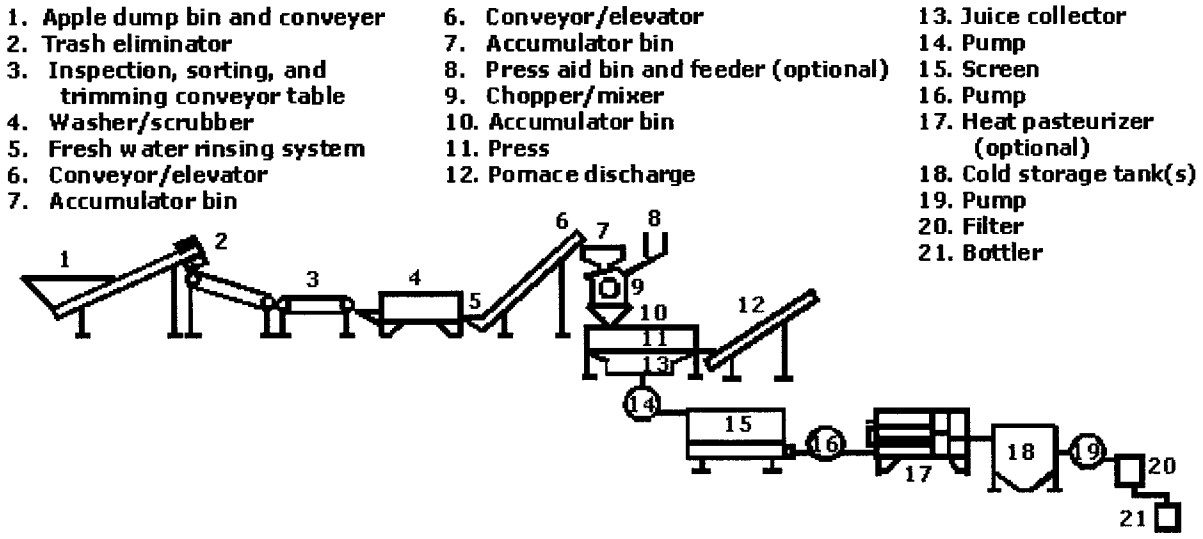


Figure 1. Ideal system of operations for cider production (PennState, 2003).

Many parameters are used to determine the suitability of apples for the production of apple cider. Mature apples have been demonstrated to be the best in terms of aroma and flavor characteristics. Blending of cider apples with high acid and low acid contents is also

important since the combination of similar apple cultivars may yield an undesirable cider. Maturity and flavor profiles, however, are not the only criteria that determine what apple cultivars are suitable for cider production. The yield of juice from an apple cultivar is also quite important to its inclusion in an efficient cider making process.

When following ideal processing conditions, the yield of apple cider from fresh apples can vary between 70 to 83 percent by weight. According to the Pennsylvania Tree Fruit Production Guide (PennState, 2003), cider yield may be lower when the apples which are pressed are overripe, when apples are ground to a particle size larger than 3/8-inch, when using an inefficient or defective press, or when the depth of chopped apples in a press exceeds 2 inches.

Poll (1981) assessed eighteen apple cultivars for suitability in juice production using both chemical and organoleptic analyses. In addition to degree of ripeness based on seed color, firmness and total acid content, juice production was estimated using a centrifuge. Chemical analyses included soluble solids, total polyphenol content, and degree of browning by spectrophotometric absorption at 420 nm. Filtered and pasteurized juice was stored at 10°C for six months before sensory and chemical analyses were completed. Poll's evaluation (1981) found that overall aroma and taste was strongly correlated to fruit aroma using regression models. Intensity of fruit aroma, determined by sensory analysis, had a strong influence on the overall quality of apple juice. Variation between two different years of study was noted since total polyphenol content was quite variable and most cultivars had lower acid contents in 1978 than in 1977. All eighteen apple cultivars were summarized in terms of organoleptic quality, acid content, juice yield, polyphenol content and other notable characteristics.

Phenolic content can be affected during common cider processing methods. According to Lea and Timberlake (1978), oxidation of phenolics involves the irreversible binding of phenolics to surrounding apple pomace with consequent loss in characteristic bitterness and/or astringency during milling or before pressing steps. In addition, enzymatic oxidation of phenolics into quinones and further polymerization can produce browning compounds, hazes or even sedimentation in apple cider (Mangas et al., 1999). If apples with high polyphenol content are chosen for cider production, the levels present in a final product may be quite less depending on losses during production methods.

The environment to which phenolic compounds are exposed during apple cider production can also affect antioxidant activity. According to Xuetong and Thayer (2002), ionizing radiation increased antioxidant activity, quantified on a ferric-reducing basis, of apple juice under specified dosage and temperature conditions. Furthermore, the exclusion of oxygen by nitrogen flushing also resulted in increased antioxidant power.

The process of cider production normally involves forceful grinding or pulverization of whole apples into a puree. Subsequent pressing of the puree may create a substance quite different than that of whole apples. Enzymatic cleavage of esters can occur during disruption of apple tissue (Williams et al., 1981). Furthermore, oxidation products of fatty acids can serve as precursors for new flavor products, i.e. aldehydes and alcohols. Apple maceration also induces the release of methanol from pectin by action of the enzyme pectin methyl esterase. Crushing of apples has been shown to increase the synthesis of additional alcohols such as 3-methyl-1-butanol (isoamyl alcohol), 2-phenyl ethanol (2-phenyl alcohol), propanol, butanol, and 2-methyl-1-propanol (iso-butanol) (Vidrih and Hribar, 1999). Removal of insoluble solids through filtration was found to reduce the synthesis of certain alcohols, but

since cider can not be selectively filtered this application is not possible. Fermentation and alcohol production, therefore, must be controlled by other means.

Natural growing and ripening processes can be responsible for the synthesis of both desirable and undesirable alcohols in apples. Following apple cider pressing, yeast and bacteria can also metabolize sugars and acids to produce alcohol. Fermentation and alcohol synthesis can often be monitored by a decrease in glucose, fructose and total soluble solids. Uncontrolled fermentation and undesirable flavor development in apple cider, however, is often accelerated with the use of outdated production methods. Wooden mills, wild microflora, and uncontrolled temperature conditions specifically trigger unpleasant-flavored cider. New manufacturing practices that produce cider under sanitary conditions and monitored temperatures, with the use of select apple cultivars, may initiate the production of more desirable flavored apple cider (Keller et al., 2002).

During fermentation, yeasts metabolize sugars to produce alcohol and carbon dioxide (Jay, 2000). In one study, more than 500 strains of yeast were isolated from apples, leaves and soil in 23 orchards throughout Canada. Flavor of apple cider was dependent on the types of yeast present (Emard, 1974). Distinguishing strains include *Saccharomyces cervisiae* as a strong fermentative organism (Deak and Beuchat, 1996), *Hanseniaspora uvarum* as being responsible for off-flavor development, and *Saccharomyces ludwigii* as a major cause of spoilage in bottled cider (Carr, 1984).

Regulations to Improve the Safety of Apple Cider

Increased safety is always a major concern for the production and handing of apple cider. As a result of previous foodborne illnesses caused by apple cider, the FDA mandated that all apple cider producers must apply HACCP (Hazard Analysis Critical Control Points)

before January 20, 2004 (FDA, CFR 2001). In relation to HACCP, the importance of GMPs (Good Manufacturing Practices) and SSOPs (Sanitation Standard Operating Procedures) was examined by comparing the quality of apple cider produced under good versus inadequate processing environments. Visibly damaged and moldy apples were processed and both frequent and infrequent cleaning and sanitation practices were employed. A high level of microorganisms in juice resulted from apples of low microbial content because of unsanitary equipment. This demonstrates the impact equipment can have on the contamination of apple cider during processing. In addition, uncovered collection tanks can also contribute to additional contamination from airborne microorganisms (Keller et al., 2002).

Manufacturing processes can vary greatly for each apple cider producer. According to a survey conducted in the 1990's by Senkel et al. (1999), all eleven cider producers reviewed in Maryland did follow control strategies. None of the producers surveyed had specific GMPs, SSOPs or monitored records, and only one producer had an actual HACCP plan. In the survey, the producers were asked about the source of cider apples, whether dropped apples were crushed for cider, methods to remove filth, time/temperature combinations of pasteurization (if applied), concentration of preservatives (if used), and other questions. Furthermore, a safety workshop with industry, regulatory and educational representatives was conducted to inform apple cider producers of the benefits of HACCP implementation. GMP and SSOP procedures were explained as an important prerequisite for three separate cider HACCP processes, pasteurized cider, unpasteurized cider with tree-fruit, and unpasteurized cider with windfall fruit. Illustrations for apple cider production were developed.

Each HACCP model designed for Maryland cider producers was provided with specific critical control points, preventative measures, critical limits, monitoring practices, corrective actions, record keeping practices, and verification. To evaluate the effectiveness of HACCP implementation, microbiological data from 1993 to 1996 (before HACCP) was compared to that from 1997 to 1998. During the 1997-1998 season, all cider producers used at least one preventative measure that was not previously used. Overall sanitation practices improved since several producers utilized additional apple and cider treatments such as the exclusion of dropped apples, sanitation of apples before milling, and pasteurization equipment. The occurrence of cross-contamination was reduced as a result of more frequent cleaning and sanitizing of equipment, more frequent hand-washing, or the use of aprons and/or gloves by operators. Sampling of fresh cider indicated a 0.50 log count/g decrease in standard plate counts and 0.40 log MPN(most probable number)/100g decrease in total coliforms from 1993 to 1996 (Senkel et al, 1999). Other factors, such as variation in weather and apple types, could have contributed to a decline in microbial loads.

Another study compared microbial loads between two years of cider production in the state of Iowa. It was expected that HACCP implementation during the 2000-2001 season would reduce microbial loads in comparison to the 1999-2000 season. While results of the study did not support a significant difference in microorganism populations between the two seasons, improvements made in sanitation and manufacturing methods are thought to contribute to an overall increase in apple cider quality and safety (Cummins et al., 2002).

Even though cider producers became more aware of production controls as a result of the safety workshop in the Maryland study, many problems were identified following HACCP improvements. Many of the producers involved did not have sufficient time to

develop customized HACCP plans (based on their operation) and only a basic plan was available to them (Senkel, 1999).

On the other hand, a priority made in sanitation of equipment versus sanitation of apples could have been the cause of varied results. For example, a facility that brushes, washes and sanitizes apples before the milling process could have introduced contamination during processing if the equipment is not properly sanitized. In some instances, washing and rinsing apples prior to milling is not always effective since the interior of apples is not penetrated, microorganisms may be resistant to certain sanitizers, and wash water may be contaminated. A combined approach entailing all HACCP, GMP, SOPs, and sanitation practices should be applied in order to ensure cider safety (Senkel et al., 1999). At the same time, it is important to producers that processing methods remain economical and efficient.

A more recent publication summarized the manufacture of apple cider in Ontario, Canada by conducting a telephone survey of fifteen producers (Luedtke and Powell, 2002). Thirty-two questions covered aspects of foodborne illness awareness, cider sales, orchard and facility management, processing and storage methods, cider testing, safety plans and other topics. Most of the output from the survey was positive. Since the producers were aware of the *E. coli* risk associated with using dropped apples in cider, producers who used dropped apples did so in conjunction with pasteurization. All fifteen producers stated that they cleaned and sanitized equipment after each batch in order to prevent contamination from residual fruit. Another encouraging note was that all producers who sold to large retail stores pasteurized their cider. Reasons for pasteurizing include consumer or retail store demands and market leverage.

The survey conducted in Canada also obtained some negative feedback concerning implementation of processing procedures. Processors that did not pasteurize suggested that consumers insisted on unpasteurized cider because of flavor losses caused by thermal pasteurization. Others said they could not afford the costs of pasteurization and would be forced out of business if pasteurization were mandated. Furthermore, many producers identified critical control points but did not implement HACCP plans because of extensive documentation that is required. Some producers mentioned that additional literature and background information would be needed in order to carry out a HACCP plan (Luedtke and Powell, 2002).

Government regulations and enforcement of good manufacturing practices are minimal in Canada compared to the United States. Since it is not mandatory to label unpasteurized cider in Canada, many producers sell cider without such label statements. For this reason, Luedtke and Powell (2002) stated it is important that consumers, governmental agencies and the cider industry collaborate to reduce the risk of foodborne illness by risk assessment, management and communication efforts.

Processing Steps to Improve Safety of Apple Cider

Regardless of the effectiveness of GMPs, SOPs and HACCP regulations, apple cider sold in grocery stores must be deemed safe to consume. The FDA regulation requiring HACCP implementation does not identify specific processing methods to cause a 5-$\log_{10}$ reduction in pathogen populations. Therefore, many techniques such as the addition of preservatives or application of heat pasteurization, ultraviolet radiation (UV), or electron beam irradiation can be used.

Addition of preservatives

Preservatives, such as potassium sorbate and sodium benzoate, may be added during the processing of apple cider to increase shelf-life (Luedtke and Powell, 2002). Sorbate is an effective inhibitor of yeasts and molds since a low pH environment, found in apple products, allows inhibition of various microorganisms' enzyme systems (Baroody and McLellan, 1986). Based on certain laboratory studies, though, potassium sorbate (0.1%) was found to have little effect on *E. coli* 0157:H7, while sodium benzoate allowed the pathogen to survive under refrigeration temperatures for three weeks (Wright et al., 2000). For this reason, preservatives are often used to extend shelf-life and not for the elimination of pathogens.

Ingham and Schoeller (2002) considered multi-step interventions against *E. coli* 0157:H7 using both temperature and chemical (sodium benzoate and potassium sorbate) means of control for pressed apple cider. The experiment involved a combination of three steps: a "warm hold" phase, the addition of a preservative (sodium benzoate and/or potassium sorbate), and a freeze/thaw cycle. The warm hold consisted of holding cider between 25-35°C for six hours which was thought to increase the lethality of *E. coli* by organic acids. The freeze/thaw cycle held cider at -20°C for 48 hours, followed by 4°C for 5 hours. Microbial counts were completed thereafter. Lethality can be linked to cider pH since cider at a pH of 4.1 had approximately two orders less magnitude of lethality using the multi-step intervention than cider at a pH of 3.3 (Ingham and Schoeller, 2002).

Consumer acceptance of apple cider is also related to preservative concentration. A sensory panel using a 7-point hedonic scale showed more dislike (one point degree) for cider with 0.1% of preservative compared to cider with 0.05% preservative (sodium benzoate and/or potassium sorbate). Even though a multi-step process intervention may provide a 5-

log reduction in pathogens, results were inconsistent under such conditions since sample variability is complicated in terms of varied pH and °Brix. The temperature control and monitoring of such conditions is complicated and not very practical for commercial apple cider production (Ingham and Schoeller, 2002).

A study conducted by Comes and Beelman (2002) treated apple cider with GRAS (generally recognized as safe) acidulants and preservatives (potassium sorbate and sodium benzoate) to achieve a 5-$\log_{10}$ reduction in *E. coli* O157:H7 populations in apple cider. Following acid and/or preservative treatment, samples were inoculated with *E. coli* and subjected to mild heat treatments before refrigerated storage. While citric and malic acid had no effect, 0.1% fumaric acid alone had a significant effect on pathogen reduction. The combination which achieved a 5-$\log_{10}$ reduction in cider was the addition of 0.15% fumaric acid and 0.05% sodium benzoate followed by a 6-hour holding period at 25°C (Comes and Beelman, 2002). While consumers found the preservative cider to be acceptable, the final pH was as low as 3.2 and off-flavors due to sodium benzoate become more apparent.

The stability of sorbate in food systems (irradiation) is a concern in the application of this additive to extend the shelf-life of apple cider. Sorbic acid is known to be stable in the pure form, but stability is altered in aqueous solutions. According to Gerschenson et al. (1986), sorbic acid has greater losses in pH 3.5 model systems compared to those at pH 4.5, and its destruction follows first-order kinetics. In addition to pH, degradation of sorbic acid depends on time and temperature conditions and amounts other compounds present. According to Arya (1980), sodium chloride, potassium chloride, sucrose and trace metal ions (Cu^{2+}, Fe^{2+}, Mn^{2+}) decrease the rate of sorbic acid degradation while amino acids (except histidine and arginine), acetic acid, glycerol and other salts increase the rate of degradation.

Pasteurization

Pasteurization has been widely utilized for cider preservation since it destroys all disease-causing pathogens and reduces the number of spoilage microorganisms (Kozempel et al., 1998). The cost of pasteurization is minimal in relation to the retail price of apple cider. An estimate suggests that cider costs $1.00/L and pasteurization accounts for less than a $0.01/L increase. Factors concerning the pasteurization facility, i.e. plant size, energy use, operation hours, post-pasteurization handling and time/temperature combinations may alter the actual cost of pasteurization services (Kozempel et al., 1998).

The effects of pasteurization can vary according to the time/temperature variation of the treatment but overall effects on apple cider flavor have been reported. Based on a study by Wang et al. (2003), pasteurized cider has less apple flavor than untreated cider. Fischer and Golden (1998) reported the adverse effects on apple cider color, flavor and viscosity following pasteurization. Additional studies support an increase in cooked aromas and/or flavors as fruity characteristics decreased following exposure or storage of apple cider at elevated temperatures (Poll and Flink, 1983; Poll, 1983; Poll, 1985; Bettini, 1998). The conclusions of such studies were based on sensory and instrumental evaluations.

According to Piyasena et al. (2002), the physical and chemical properties of apple cider can vary with the blend of apples used. Furthermore, the thermal resistance of microorganisms is strongly influenced by certain characteristics (i.e. the pH, type of acid, sugar content and solids level) of the heating medium. A universal pasteurization process for the manufacture of safe apple cider, therefore, may not be practical, and cider composition should be considered when developing a thermal inactivation process for foodborne pathogens.

Ultraviolet light penetration

Light energy is classified on a spectrum in which wavelength is measured using a nanometer (nm) scale. The lower end of the scale has the shortest wavelength. Cosmic, gamma, x-rays and "C" band UV are all classified short-wave energy. Visible light is between 400 and 700 nm, infrared light is between 800 to 1400 nm, and radio waves are in the 1400 to 2200 nm range. More specifically, ultraviolet light is from about 100 to 400 nm, with three categories, "A," "B" and "C." Short-wave UV, called "C" band (100 – 280 nm), is known as UVC. UVC radiation typically gets screened from the sun by ozone in the atmosphere before reaching the earth. Useful UVC is entirely manmade, found in today's low-pressure UVC lamps.

With initial exposure, UVC has properties that alter the cells of living tissue, particularly microorganisms. UVC radiation triggers the formation of peptide bonds between certain nucleic acids in DNA molecules. This renders bacteria, viruses and molds harmless by restricting reproduction. If the germ cells are exposed for longer periods, they start breaking down to the molecular level (carbon, oxygen, hydrogen, nitrogen ions, etc.). Within the C bandwidth of UV light, the most effective range as a germicidal or mutagenic agent is between 240 and 280 nm (Marquenie et al., 2003). The effectiveness of UVC light, however, can vary with commodity type, maturity and other factors.

UVC light has been studied as a control for postharvest decay of whole apples (Wilson et al., 1997). Ultraviolet light has also been investigated as a processing method for apple cider since microbial populations can be greatly reduced by specially designed UV lamps. Microbial kill was affected by cider clarity, UV exposure time, and the presence of potassium sorbate. Viable microbial counts were reduced 99% by 40 sec of UV irradiation in

a thin film of flowing cider without affecting the flavor (Harrington and Hills, 1968). Ultraviolet treatment, however, is not always efficient for apple cider processing. The optical density, i.e. cloudiness, of cider can interfere with the penetration of light and bacterial numbers are not efficiently reduced (Senkel et al., 1999).

Irradiation

The process of irradiation has greatly evolved over the last century. Around the beginning of the 20th century, three different types of radiation, alpha (α), beta (β) and gamma (γ) rays, were discovered (Josephson, 1983). By 1905, both British and American patents were proposed for the use of irradiation to kill bacteria in food. In the form of an amendment to the Food, Drug and Cosmetic Act of 1958, irradiation was defined as a food additive. Shortly thereafter, the United States Army began a program on food irradiation using Cobalt-60, Cesium-137 and an electron accelerator. Today, labeling with the radura symbol illustrates the 'treated with irradiation' statement.

Two major sources of ionizing energy exist, radionucleotides and machine sources. Radionucleotides, such as Cobalt-60 and Cesium-137, emit gamma rays by way of a nuclear reactor. Machine sources are X-rays and high speed electrons. Electron beam accelerators emit electrons into an evacuated chamber. These electrons are attracted to a high positive electric potential and are focused onto a narrow beam. This electron beam usually passes between an electromagnet with a changing magnetic field, causing the beam of electrons to pass from side to side. Electrons are accelerated to a set quantity of volts and hold enough energy to penetrate a food product.

Gamma rays (photons with no mass) have energies from 0.66 to 1.3 MeV (million electron volts), allowing deep penetration into foods. Absorption of gamma ray energy

decreases exponentially as it travels through the food. High speed electrons, on the other hand, are generated and accelerated to energies up to 10 MeV, which is the highest electron energy permitted for foods. Electrons have a small mass and lose energy as they penetrate a food source, which limits penetration into foods. With 10 MeV electrons, depth of penetration into foods (densities similar to water) is limited to about 3.7 cm with one-sided exposure (Olson, 2003). Eventually, high speed electrons are captured by positive ions as they are reflected or absorbed.

A conveyor system allows a uniform dose of high speed electrons to be released. As the high speed electrons travel, energy is transferred and atoms become unstable. Electrons from an innermost, stable orbital may be ejected, leaving the atom positively charged and chemically reactive. The effects of this ionizing energy on atoms and molecules can vary. Molecules can be chemically altered since the outer shell of electrons control how molecules react. Dose measurements (i.e. the total amount of absorbed energy) depends on a number of factors such as energy source, strength and exposure time and food mass, bulk density and thickness. Dose is defined as the quantity of energy absorbed during exposure [1gray (Gy) = 100 rad = 1 Joule/kg product] (Buchalla, 1993). Dosimetry is the process of measuring radiation dose. Quality control procedures exist for the dosimetry of each commodity, as well as environmental humidity, temperature and lighting conditions.

Fruits and vegetables have been irradiated for sprout inhibition, alteration of ripening rates, disinfestations, and/or control of postharvest pathogens to ultimately increase product shelf-life (Thayer and Rajkowski, 1999). One study by Boylston et al. (2002) evaluated the effects of irradiation on sensory attributes of papayas, rambutans and oranges. The effect of irradiation varied for each fruit. The aroma and flavor of irradiated papayas was less intense

than unirradiated papayas while the aroma and flavor of irradiated rambutans was more intense than unirradiated samples. No significant differences were found between irradiated and unirradiated oranges. It was also noted that irradiation did not significantly affect soluble solids or titratable acidity. Overall, irradiation of the three tropical fruits showed no significant differences in nutrient and chemical quality compared to control fruit, with only minor changes in sensory quality as a result of irradiation (Boylston et al., 2002).

Additional studies have focused on the irradiation of fruit juices as a non-thermal treatment to eliminate pathogens while preserving sensory attributes. A study by Foley et al. (2002) employed gamma-irradiation to fresh orange juice to determine doses necessary for 5-$\log_{10}$ reductions in *L. monocytogenes* (2.4kGy) and *Salmonella enterica* (2.65kGy). While irradiation treatment was effective in destroying pathogens, the development of off-flavors was noticeable at levels as low as 0.7kGy (Foley et al., 2002). Since orange juice is quite sensitive to the effects of irradiation, differences in composition of apple cider and orange juice can account for differences in irradiation effects between the two food products.

For FDA approval, "a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" must be established (FDA, 2003). In 1981, the FDA supported irradiation as a food additive (with absorbed doses up to 10 kGy) because the process generates radiolytic products and affects the characteristics of a food. Soon thereafter, ionizing radiation was investigated as a non-thermal method to cause a 5-$\log_{10}$ reduction of human pathogens in apple cider since it can inactivate food-borne pathogens and reduce spoilage. The necessary dose is dependent on pathogen species and strain but a 5-$\log_{10}$ reduction is evident following irradiation doses between 1-3.55 kGy

(Xuetong and Thayer, 2002). According to Wang et al. (2001), a 2.17 kGy dose of electron beam irradiation is sufficient to obtain a 5-$\log_{10}$ reduction of *E. coli* 0157:H7 in apple cider.

Radiological, toxicological, microbiological, nutritional and sensory alterations are possible as a result of ionizing radiation. For this reason, detection of irradiated food and the effects of irradiation on food products have been investigated. Different physical, chemical and biological methods such as electron paramagnetic resonance, luminescence and changes in DNA and microflora show specific alterations as a result of ionizing energy penetration. Variation between individual foods, however, makes it impossible to use one single method to detect specific effects of irradiation (Glidewell et al., 1993). The following questions remain: Are internal repair mechanisms destroyed or altered by irradiation? Does ionizing irradiation cause vitamin depletion? Are radical cations which are available for formation of secondary radicals created during the process of irradiation? Does irradiation allow the survival of bacterial toxins?

According to an article written by Wolke (2002), much of the opposition to food irradiation is based on misunderstandings and resulting fears. Most of the general public recognizes the positive effects of food irradiation on the reduction of food-borne illnesses and extension of shelf-life. The fear of radiation, however, stems from the notion that irradiated food becomes radioactive. Approved doses for electron beam irradiation create radiolytic products, but do not have enough energy to make anything radioactive (Hayes et al., 2002). The Food and Drug Administration, Department of Agriculture, Centers for Disease Control and Prevention, Institute of Food Technologists, American Medical Association, and World Health Organization have all approved the safety of various forms of irradiated foods (Wolke, 2002).

The effect of ionizing radiation on the stability of sorbic acid in food systems has also been investigated. According to Thaker and Arya (1993), γ-irradiation caused degradation of sorbic acid in orange juice and mango pulp. Sorbic acid is expected to scavenge free radicals that are produced during irradiation. In irradiated orange juice, 0.1% sorbic acid decreased ascorbic acid loss, decreased the rate of browning and completely inhibited off-flavors (Thaker and Arya, 1993). It is unknown whether the same effects would take place in apple cider as those exhibited in orange juice. Since the process of cider pressing causes a loss in ascorbic acid, this compound would have to be re-added to cider following production.

Food Packaging

Before the wide utilization of plastics, metal and glass were the primary materials used for food packaging. Glass has a prime advantage of being chemically inert and airtight, but major disadvantages include susceptibility to breakage and heaviness, which contributes to increased transportation costs. In the past, a concern of iron, tin and lead was present when these metals were found in food products that came in contact with the packaging materials (Askar, 1999b). Today, a concern with many aspects of plastic packaging materials exists.

Plastics used for packaging are normally constructed of hydrocarbon chain polymers such as ethylene, propylene or styrene. Polyethylene is classified as a polyolefin while polystyrene is a styrenic resin and nylon is a polyamide (Appendix B). Low-density polyethylene (LDPE), polystyrene (PS) and nylon-6 (N6) are three types of packaging materials widely applied to food products. LDPE film was originally developed for sliced bread storage. Today, countless forms of LDPE exist for the use of sandwich bags, grocery bags, shrink wrapping and other film applications. Nylon films have been used for vacuum

packaging meats, cheeses and frozen foods because of low gas permeability and low odor transmission properties. Polystyrene is formed into a variety of tubs, trays, cups and other containers of various shapes and sizes (Briston and Katan, 1988).

Three possible modes of transport exist for the mass transfer of packaging matter or materials into food products. Migration is the transfer of low molecular weight molecules from packaging material into the food, permeation involves the transfer of molecules (odors, flavor components, and gases) through packaging material, and absorption is the actual incorporation of food components by a packaging material (Passy, 1983; Sizer et al., 1988). Each packaging material has unique permeability properties to gases, vapors and liquids, which can limit the transfer between packaging and food products (Hopfenberg, 1974). A few of the parameters used to measure the permeability or stability of packaging materials include (1) the gain of oxygen from ambient air; (2) the loss of carbon dioxide from carbonated beverages; (3) the loss of moisture from foods; (4) the gain of moisture from ambient air; (5) the loss of ethanol from alcoholic beverages; (6) the resistance to oil migration; and (7) the loss of volatile flavor compounds (Salame, 1974).

A study by Askar (1999b) reported the interactions of fruit juices with packaging materials. Direct migration of manufacturing compounds (residual solvents, monomers, stabilizers, plasticizers, antioxidants, and ultraviolet-light blockers), absorption of flavor compounds, and transmission of light or oxygen are the main points of his study that can cause such interactions. Low transport and storage temperatures can minimize color loss, flavor deterioration, and migration from plastic contact materials in fruit juices (Askar, 1999b).

Irradiation and packaging

Packaging materials intentionally get exposed to irradiation in certain instances such as manufacturing, preservation/sterilization, and microwave cooking. Electron beam irradiation is often applied to polyethylene to aid in polymerization and cross-linking, thus reducing gas and water transmission rates and obtaining some thermoset properties. Beta and gamma irradiation are sometimes used in aseptic packaging systems. Irradiation can be an alternative to ethylene oxide for the sterilization of packaging materials prior to packaging with food products. Food products can also be preserved with irradiation while they are already packaged. Finally, microwave cooking can involve heat conduction from the food with packaging material (Briston and Katan, 1988).

The formation of gases such as hydrogen (H_2), carbon dioxide (CO_2), carbon monoxide (CO), and methane (CH_4), or volatiles such as hydrocarbons, alcohols, aldehydes, ketones and carboxylic acids result from the irradiation of polymers. Simultaneous scission and cross-linking of polymeric chains can ultimately affect changes in physical properties. In addition, the formation of unsaturated bonds and presence of oxygen can encourage the formation of oxidation products such as peroxide, alcohols, carbonyls, CO, CO_2 and other oxygen-containing compounds. The extent of radiation-induced changes depends on many factors such as the type of polymer, processing exposure and irradiation conditions (Buchalla, 1993; Azuma et al., 1983).

Irradiation-induced changes to packaging materials must also be considered since the extent of transfer or contamination is important for toxicology and organoleptic quality and can vary with packaging material, packaging environment, irradiation dose, and food type. For example, metal (aluminum and tin) and glass containers are quite resistant to irradiation

with only slight changes observed in color. Paper, cardboard or plastics, however, are not as tolerant to irradiation. Paper and cardboard can demonstrate a loss in mechanical strength while plastics can exhibit cross-linking, cleavage or modifications in chemical structure (Buchalla, 1993).

A study by Azuma et al. (1984) used GC-MS to identify volatiles from electron beam irradiated polyethylene film. Hydrocarbons, aldehydes, ketones, and carboxylic acids comprised a majority of the volatiles collected from irradiated polyethylene film. The settings and conditions of irradiation affected the amount of such volatile compounds. Based on the results of this study, the amount of carbonyl components (carboxylic acids, aldehydes and ketones) that result from the irradiation of low-density polyethylene film (LDPE) was much higher in the presence of atmospheric oxygen than when the oxygen level was more limited ($\leq$1%). Removal of oxygen caused a restraint in the available oxygen for carbonyl formation. Overall, restriction of total volatiles from irradiated LDPE film results from low oxygen concentration, low radiation energy and high beam currents (Azuma et al., 1984).

A relationship between flavor compounds and irradiation of polymer films is necessary to explain the possible affects of such processes. Studies by Matsui et al. (1990; 1992) used physical measurements such as film crystallinity, melting point, tensile strength and degree of cross-linking to determine changes caused by irradiation. Parameters such as film diffusion, permeation and solubility characteristics were used to study the compatibility of polymers to a model flavor solution. Migration of flavor compounds, most likely caused by alterations in film characteristics, took place following electron beam irradiation. LDPE (low-density polyethylene) has inferior gas barrier properties, and the sorption of flavor compounds is more distinct in LDPE than in other films (Matsui et al., 1992). It is thought

that the deterioration of flavors in storage can be attributed to packaging materials such as LDPE. The effect of irradiation on packaging materials and subsequent migration of compounds to and from food material, therefore, is quite important to the safety and quality of irradiated apple cider.

Additional studies have been completed to provide the basis for safety evaluation of plastic packaging materials used for food irradiation. Since foods are often irradiated in their final packages, literature published by Buchalla et al. (1993) helped summarize the effects of ionizing radiation on chemical and physical changes caused in plastic food packaging materials. In contrast to other literature, Buchalla reported that irradiation (0-8 kGy) does not cause changes in gas permeability or crystallinity properties of LDPE, HDPE (high-density polyethylene), polypropylene, polyethylene terephthalate, and poly(vinly chloride) packaging materials. Therefore, it is possible that the characteristics of packaging materials are not altered as a result of irradiation but can influence the migration of compounds between packaging materials and food products.

A study by Lopez-Gonzalez (2000) conducted sensory evaluation on ground beef patties packages in three plastic polymers and exposed sample to gamma or electron beam irradiation. The films which were tested were 1) a nylon/polyethylene bag, 2) a saran/ polyester/polyethylene bag, and 3) a saran film overwrap. Based on the findings of the experiment, irradiation treatment (2kGy) did not provide statistical differences in sensory data. Packaging material or irradiation source did not affect the quality of the patties.

Techniques for Flavor Analysis

Sensory evaluation, volatile flavor analysis and analytical measurements (soluble solids, pH and titratable acidity) are helpful in monitoring flavor quality of food products.

Sensory evaluation is based on the use of human senses while volatile flavor analysis and analytical measurements involve instrumentation.

Sensory evaluation

Sensory evaluation is helpful in identifying overall differences in food products with the help of human subjects, i.e. consumers or trained panelists. Based on work using descriptive analysis, trained panelists detected a more "musty" (Yulianti, 2003) or "cardboard-like" (Boylston, 2003) off-flavor in irradiated cider with sorbate and pasteurized cider with sorbate. However, in the absence of sorbate, a significant difference was not detected between irradiated and pasteurized cider. It was concluded that this off-flavor was a result of irradiation effects on sorbate.

Consumer tests conducted by Yulianti (2003) also compared irradiated and pasteurized apple cider. Consumers in central Iowa detected a difference between irradiated and pasteurized cider, but both samples were liked to the same degree. In the absence of sorbate, consumers could not distinguish a difference between irradiated and pasteurized samples. In the presence of sorbate, however, a significant difference was found between irradiated and pasteurized apple cider.

Sensory testing is quite useful in evaluating overall flavor profiles of foods. Sensory studies have found irradiated apple cider to have a less-intense apple flavor than unirradiated (raw) apple cider (Yulianti, 2003; Wang, 2003). However, these results do not provide insight to individual flavor compounds or analytical measurements. It would be helpful to complement sensory analysis with instrumental methods of measurement such as volatile flavor analysis and analytical measurements.

Volatile flavor analysis

Identification and quantification of flavor compounds is frequently done on a basis of volatile analysis, which involves isolation, purification and/or concentration from the surrounding medium. Steam distillation, solid-liquid extraction, purge and trap extraction, simultaneous distillation-extraction and batch and continuous solvent extraction methods have positive and negative qualities concerning techniques and recovery. Concurrent distillation-extraction is less time-consuming than liquid-liquid extraction, but artifact formation is possible with extensive thermal exposure. Distillation and liquid-liquid extraction are great for examining aroma compounds, but are time-consuming (Watada et al., 1981) and the stability of the emulsion may cause difficult phase separation. Furthermore, static headspace technology is very practical for food and beverage aromas, yet it has low sensitivity with respect to trace volatile compounds (Mangas et al., 1996).

Solid-phase microextraction (SPME) is a method for concentrating volatile or nonvolatile compounds in liquid samples or headspace which eliminates the need for solvents or complicated equipment. SPME is faster and much less labor intensive than liquid-liquid extraction or solid phase extraction and requires small amounts of sample. According to Matich et al. (1996), SPME offers rapid and nonintrusive quantitative sampling of apple volatiles, especially for low molecular weight compounds which equilibriate rapidly.

SPME can be used to detect flavors in both solid and liquid foods and a wide range of volatile and semivolatile compounds can be analyzed and detected. Dynamic headspace using purge and trap techniques is limited to more volatile compounds and can only be used for liquid foods (Supelco, 1998). Solid-phase microextraction (SPME) techniques have been developed specifically for headspace analysis of volatile flavor compounds (Roberts et al.,

2000). More specific methods are economical and appropriate for quantitative measure of flavor compounds in cider using GC-MS (Gas Chromatography-Mass Spectrometry). According to Mangas et al. (1996), acceptable recoveries and accuracy of alcohols, esters, lactones and phenols are noted with the use of solid-phase microextraction.

SPME involves simple adsorption/desorption techniques by use of a fused silica fiber. As a plunger moves the fiber into and out of a hollow needle, organic compounds adsorb onto the coating of an exposed fiber and the needle. Following equilibration, the fiber can be directly injected onto a GC column or HPLC machine. Selectivity of compounds is based on the affinity of such organic compounds to the fiber coating and various forms of coating thickness and polymer polarity are available. A polar SPME fiber and GC column is more effective in extracting polar compounds from a sample, just as a non-polar fiber and column is more efficient in extracting non-polar compounds.

At least seven commercial SPME fiber types are available. Examples of polar fibers include 85μm *polyacrylate (PA)*, 65μm Carbowax/*DVB* (*divinylbenzene)*, and 50μm Carbowax/*template resin* while a non-polar fiber is 100μm, 30μm, 7μm poly-dimethylsiloxane (PDMS). Examples of bi-polar fibers include 65μm PDMS/*divinyl-benzene* (*DVB*), 75μm *Carboxen*/PDMS and 50/30μm *DVB*/*Carboxen*/PDMS (μm = fiber coating thickness; in italics = porous absorbants; not in italics = commercial GC phases). For flavor analysis, the 50/30μm *DVB*/*Carboxen*/PDMS fiber has advantages since it allows for absorption of a wide range of analyte polarities such as volatile and semivolatile compounds and some gases. It is widely applied for the volatile flavor analysis of foods, especially beverages. The 75μm *Carboxen*/PDMS fiber does not adsorb semivolatiles while the 65μm PDMS/*DVB* and 100μm PDMS does not adsorb gases (Supelco, 1998).

Analytical measurements

Flavor quality can also be monitored on a basis of a sugar to acid ratio. This ratio can be directly related to sensory properties of sweetness and tartness. Apple cider is comprised of sugars such as sucrose and organic acids such as malic acid. Sucrose and malic acid levels can be measured using instrumental analyses. Therefore, the sensory sweetness and tartness can be directly related to analytical measurements.

Volatile flavor analysis is helpful for identifying and quantifying specific flavor compounds in apple cider. However, differences detected in volatile flavor compounds can not be directly related to sensory analysis. Ideally, the incorporation of sensory analysis, volatile flavor analysis and analytical measurements would serve as necessary evaluations for the flavor quality of apple cider even though limitations do exist for each method.

Experiment Objectives

The objective of this study was to evaluate the flavor characteristics of irradiated apple cider during storage. Flavor characteristics were monitored by use of volatile flavor analysis and analytical measurements (soluble solids, pH and titratable acidity). Specific objectives of Experiment I were to determine the effects of packaging material and sorbate addition on the retention of characteristic flavors in irradiated apple cider during three weeks of refrigerated storage. Apple cider irradiated in three plastic polymers, polystyrene (PS), low-density polyethylene (LDPE) or nylon-6 (N6), was compared to unirradiated cider packaged and stored in glass containers. Specific objectives of Experiment II were to determine the effects of gaseous environment and sorbate addition on the retention of characteristic flavors in irradiated apple cider during seven weeks of refrigerated storage. Unirradiated apple cider was packaged in polystyrene (PS) containers and compared to cider

irradiated and stored in PS under three gaseous environments, atmospheric air, nitrogen-flush and oxygen-flush.

Cider with (0.1%) and without (0%) potassium sorbate was evaluated initially following irradiation treatment and subsequent weeks following treatment (three weeks in Experiment I and seven weeks in Experiment II). Soluble solids, pH and titratable acidity measurements monitored quality changes caused by fermentation and other physiological reactions which take place during storage. Volatile flavor compounds of apple cider were determined and quantified using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). Experiment II also involved microbiological analyses of aerobic bacteria, yeasts and molds to monitor the growth of microorganisms throughout extended storage and determine their impact on flavor quality.

MATERIALS AND METHODS

Two separate experiments were completed to fulfill the objectives of this study. Experiment I, conducted in the Spring of 2002, focused on the effects of packaging type and sorbate addition on the flavor quality of irradiated apple cider during storage. In Experiment II, conducted during the Fall of 2002, the effects of gas environment and sorbate on flavor and microbial quality were investigated. Flavor quality, evaluated based on instrumental measurements of volatile flavor compounds, soluble solids, pH and titratable acidity, was the main emphasis for both experiments.

Ideal experimental design would have involved the comparison of unirradiated and irradiated cider in all packaging and gas environment conditions. However, in order to reduce the number of treatments, certain provisions were made. In Experiment I, the control sample was packaged in glass since this material is inert in terms of flavor migration, permeation and absorption characteristics. Therefore, the samples irradiated and stored in three different packaging materials could be compared to unirradiated cider in an inert packaging material. In experiment II, the control sample was unirradiated apple cider exposed to atmospheric air.

Experiment I: Effect of Packaging Materials and Sorbate Addition to Maintain Flavor Characteristics of Irradiated Apple Cider during Storage

Fresh cider, with (0.1%) and without (0%) potassium sorbate was packaged using four different materials. Untreated cider was placed in 200-mL glass jars while cider to be irradiated was placed into three different types of plastic containers, low-density polyethylene (LDPE) film, nylon-6 (N6) film and polystyrene (PS). The LDPE bags were 11.5 cm wide and 23 cm long (540-mL capacity), had a 2.5 mil thickness and oxygen

permeability of 276.5 cc-mil/100 in^2/24 hr @ 25°C (Nasco-Whirl Pak®, Fort Atkinson, WI). The nylon-6 bags were composed of a polyamide homopolymer, measured 15 cm wide and 20 cm long, had a 2 mil (0.002") thickness and an oxygen permeability of 2.6 cc-mil/100 in^2/ 24 hr @ 25°C (CleanFilm, Islandia, NY). The polystyrene flask containers held a volume of 250 mL (75 cm^3) and were rectangular shaped with dimensions of 2.5 cm x 9 cm x 9 cm (Costar, Cambridge, MA). Oxygen permeability was essentially zero for the polystyrene flasks. All of the packaging materials used in this experiment were pre-sterilized.

The LDPE and N6 bags were heat sealed in order to hold a cider sample of appropriate dimensions for electron beam penetration. The polystyrene containers had plug seal caps to form a closed system. The approximate depth of cider in all of the packaging materials during irradiation was $\leq$ 2.5 cm since the containers were placed on their sides. Cider was exposed to atmospheric air conditions throughout the packaging process. After irradiation treatment, volatile flavor analysis, soluble solids, pH and titratable acidity was measured initially and each week during a storage period of three weeks in refrigeration (4°C) conditions. Individual packages remained sealed until analyzed. Two replications were completed for each treatment.

Experiment I: Effect of Gas Environment and Sorbate Addition to Maintain Flavor Characteristics of Irradiated Apple Cider during Storage

Three different gaseous environments (oxygen-flush, nitrogen-flush and atmospheric air) were exposed to cider following transfer into the flasks but prior to irradiation treatment. Two hundred mL of apple cider, with (0.1%) and without (0%) potassium sorbate, was transferred into 250-mL sterile polystyrene flasks (Costar, Cambridge, MA). Atmospheric air surrounded the apple cider during transfer into each container. An oxygen-flush

environment was supplied to the cider by food-grade oxygen gas (<300 ppm carbon dioxide; <10 ppm carbon monoxide) while a nitrogen-flush environment was supplied by food-grade nitrogen gas [(>99.99% pure; <10 ppm oxygen); Linweld, Des Moines, IA]. A 20-gauge needle was used to bubble each gas at a rate of 1 psi through each 200-mL apple cider sample for 30 seconds. Plug seal caps were placed on the container immediately following bubbling of the gases to form the appropriate closed system. The third environment, atmospheric air, was designated to samples that remained open to the atmosphere during package conditioning. Atmospheric air contains approximately 78% nitrogen and 21% oxygen by volume; the remaining 1% contains trace gases such as argon, carbon dioxide, hydrogen and others. Untreated cider, i.e. cider not to be irradiated, was also exposed to atmospheric air during transfer into polystyrene flasks. Volatile flavor analysis, soluble solids, pH, titratable acidity and microbiological measurements were determined initially and at 1, 2, 3, 5, and 7 weeks after irradiation treatment and held at refrigeration (4°C) temperatures. Individual packages remained sealed until analysis. Two replications were completed for each treatment.

Preparation of Cider Samples

Fresh apple cider was obtained from a local producer during the Spring and Fall of 2002. The dominant cultivars of the cider blend were Jonathan, Red Delicious and Golden Delicious. Cider with (0.1%) and without (0%) added potassium sorbate as a preservative was collected from the same raw material blend during the same production run. For each preservative treatment, cider was mixed together to form a homogeneous blend and randomly assigned to the four treatments for each week of study. Cider was packaged and irradiated within four days of the processing date. For Experiment I, the four treatments were

unirradiated cider in glass containers and irradiated cider in polystyrene (PS), nylon-6 (N6) and low-density polyethylene (LDPE) materials. For Experiment II, the four treatments were unirradiated cider exposed to atmospheric air, and cider to be irradiated under oxygen-flush, nitrogen-flush and atmospheric air environments. In Experiment II, all of the cider was packaged in sterilized polystyrene to prevent contamination and oxygen permeation. Week 0 data represents the analyses completed directly following irradiation treatment and weeks 1, 2, 3, 5 and 7 represent the storage time following irradiation treatment.

Electron Beam Irradiation

The cider was irradiated at the Iowa State University Linear Accelerator Facility (Ames, IA) on March 1, 2002 (Experiment I-Spring 2002), and October 31 and November 7, 2002 (Experiment II-Fall 2002). Cider to be irradiated was placed in the appropriate packaging and/or gas environment and subjected to electron beam irradiation at a target dose of 2.0 kGy using an energy level of 10 MeV and power level of 10.2 kW. Alanine dosimeter pellets were attached to the top and bottom of the packages in order to measure absorbed doses (average absorbed dose = 2.22 ± 0.11 kGy). Dose rate ranged from 78.9-81.9 kGy/min while conveyor speed was set at 18.3 FPM. Untreated (raw) cider was not exposed to irradiation but did follow similar transport to and from the facility. Irradiation was conducted at room temperature without temperature control. Cider samples were stored at 4°C before and after irradiation treatment and deviated from these conditions only during irradiation treatment.

Volatile Flavor Analysis

Solid-phase microextraction (SPME) techniques were applied for the isolation and concentration of volatile flavor compounds (Supelco, Inc., Bellefonte, PA). A representative

cider sample (40g) was transferred to a 100-mL headspace bottle and sealed with a Teflon septum to prevent volatile loss. Samples were stirred using a magnetic stir bar and held in a 40°C water bath by use of a hot plate in order to increase the volatile compounds present in the sample headspace. Each sample was held in the water bath and allowed to equilibrate and absorb onto the SPME fiber [2cm-50/30μm *divinylbenzene*(DVB)/*Carboxen*/ Polydimethylsiloxane(PDMS)] for 45 minutes. The SPME fiber was removed from the sample headspace and injected onto a gas chromatograph for flavor analysis. Analyses were conducted in duplicate for each treatment.

A gas chromatograph (Model 6890, Hewlett-Packard, Inc., Wilmington, DE) equipped with a splitless injection port and flame ionization detector was used for separation of flavor compounds. Volatiles were thermally desorbed (220°C) for 3 minutes via the GC injection port onto a fused-silica capillary column (SPB-5, 30m x 0.25mm x 0.25μm film thickness, Supelco, Inc.). The column pressure was set at 124.0 kPa with a helium flow rate of 1.9 mL/min. Initial oven temperature was 30°C for 3 minutes, and four temperature ramps increased final oven temperature to 200°C. Ramp 1 was set to increase oven temperature from 30-80°C at a rate of 5°C/min. Ramp 2 increased oven temperature from 80-95°C at a rate of 4°C/min, ramp 3 increased oven temperature from 95-115°C at a rate of 5°C/min, and ramp 4 increased oven temperature from 115-200°C at a rate of 10°C/min. The detector temperature was constant at 220°C. Flow rates of detector gases were air at 400 mL/min, hydrogen at 30 mL/min and nitrogen (make-up gas) at 25 mL/min. Volatile favor standards were identified using authentic standards (Sigma-Aldrich, Milwaukee, WI; AccuStandard, Inc., New Haven, CT).

In Experiment I, volatile flavor compounds were identified and confirmed with a gas chromatograph-mass spectrometer (Trio 1000, Fisons Instruments, Danvers, MA) with a quadrupole mass analyzer. GC conditions were the same as that of the chromatographic analysis. The mass spectrometer conditions were set as the following: source electron energy at 70 eV, source electron current at 150 μA, ion source temperature at 220°C, interface temperature at 220°C, source ion repeller at 3.4 V, electron multiplier voltage at 600 V and scan range between 41 and 250 m/z. Mass spectra of the volatile flavor compounds were compared to a spectral library (NBS Library) and a flavor and fragrance database (FlavorWORKS, Flavometrics, version 2.0, Anaheim Hills, CA) for identification and verification.

In Experiment II, volatile flavor compounds were identified and confirmed with a different gas chromatograph-mass spectrometer (Micromass GCT, Waters Corp., Milford, MA) than Experiment I. The mass spectrometer conditions were set as the following: electron ionization positive (EI+) polarity, source electron energy at 70 eV, source electron current at 200 μA, ion source temperature at 180°C, source ion repeller at 0.8 V, electron multiplier voltage at 2700 V, scan range from 41 to 400 m/z, at a frequency of scanning cycle every 0.75 seconds. GC conditions were set at an initial temperature of 38°C for 1 minute, 4°C/min from 38-150°C, and 50°C/min from 150-280°C. The samples were thermally desorbed into the GC injection port in a split (100:1) mode. Mass spectra of the volatile flavor compounds were compared to a spectral library (Wiley Library) and a flavor and fragrance database (FlavorWORKS, Flavometrics, version 2.0, Anaheim Hills, CA) for identification.

Analytical: Soluble Solids, pH and Titratable Acidity

Soluble solids content was measured using a tabletop model 0-32 °Brix refractometer (Milton Roy, Ivyland, PA) with an accuracy of ±0.05% dissolved solids (Brix). Soluble solids were reported as percent sucrose. pH of the cider was recorded using a digital pH meter (Fisher Scientific, Accumet Model AB15, Pittsburgh, PA) following calibration with pH 4 and pH 7 buffer solutions. Titratable acidity was determined by titrating a sample (20 mL apple cider + 80 g water) with 0.1N NaOH to an endpoint of pH 8.2 (using the same pH as above). Titratable acidity was calculated based on malic acid as the predominant acid and was expressed as grams malic acid per 100 mL cider. Sample temperature was 20°C for each analysis. Analyses were conducted in duplicate for each sample treatment.

Microbiology

Microorganisms were directly measured from apple cider using plate count agar for aerobic bacteria and potato dextrose agar for yeast and mold counts (Experiment II only). Buffered peptone water (Difco, Detroit, MI) was sterilized and used for dilution blanks. Plate Count Agar (Difco, Standard Methods Agar) and Potato Dextrose Agar (Difco) was prepared according to label instructions and was aseptically poured into sterile, plastic Petri dishes to allow cooling and solidification. The agar dishes were stored at 4°C until inoculation took place. For each dilution prepared, duplicate samples were plated using the surface plating method. Aerobic counts were taken from the plate count agar after incubation at 36°C for 48 hours. Yeast and molds were counted from the Potato Dextrose Agar (PDA) after incubating at 24°C for 5 days. Data was recorded in colony forming units (CFU) for aerobic bacteria and yeast counts. Two replications per treatment were completed for each week of storage analysis.

Statistical Analysis

Two replications were completed for each of the treatments in both experiments. Analysis of variance and Fisher's least squares difference tests ($P<0.05$) were conducted to determine the effects of the main factors and interactions between main factors on the contents of volatile flavor compounds, soluble solids, pH and titratable acidity in both experiments using the SYSTAT statistical analysis package (version 9.01, SPSS, Inc., Chicago, IL). Experiment II also included microbiological data.

The main factors of experiment I were packaging material, sorbate addition and weeks in storage. The main factors of experiment II were gas environment, sorbate addition and weeks in storage. The effects of storage were summarized by semi-log regression slopes of log GC peak area versus week plots for each significant flavor compound. Analysis of variance and Fisher's least squares difference tests ($P<0.05$) were conducted on the slopes to determine the effects of processing treatments on changes in analytical data (soluble solids, titratable acidity) and rates of loss for volatile flavor compounds. Principal component analysis (PCA), using Varimax orthogonal rotation, was completed to examine relationships or groupings of flavor components based on treatment effects and linear regression slopes using the SYSTAT statistical analysis package (version 9.01, SPSS, Inc., Chicago, IL). PCA analysis has been applied to gas chromatograph data of raspberry volatile flavor compounds to achieve differentiation between three extraction methods (Guichard and Issanchou, 1983).

RESULTS AND DISCUSSION

This study addresses the effect of processing and storage conditions on the flavor of apple cider. The loss of characteristic flavor profiles or development of off-flavors in fruit juices can result from heat treatment, lipid oxidation, microbial growth, water-borne contaminants or contact with packaging materials. The exclusion of oxygen and use of containers that do not interact with fruit juices and are two important factors to maximize characteristic flavor retention (delay flavor loss) and achieve a reasonable shelf-life and quality (Askar, 1999a).

Two experiments investigated the effects of processing on the volatile flavor profile of apple cider. The first experiment evaluated the effects of packaging material and sorbate addition to maintain characteristic apple cider flavor during up to three weeks of refrigerated storage. The second experiment evaluated the effects of gaseous environment and sorbate addition to maintain apple cider flavor during seven weeks of storage.

Experiment I: Effect of packaging materials and sorbate addition to maintain flavor characteristics of irradiated apple cider during storage

During storage, characteristic flavor compounds of apple cider decrease as an effect of fermentation, enzymatic breakdown, or the permeation of volatile flavor compounds through packaging materials into the surrounding storage environment (Willige et al., 2001). Processing steps such as irradiation or the addition of sorbate can be applied to slow down the effects of deteriorative reactions caused by microorganisms. Furthermore, the type of packaging material used to contain apple cider during irradiation and storage may affect the retention of characteristic flavor compounds.

The objectives of Experiment I were to determine the effects of packaging material, sorbate addition and storage on the retention of characteristic flavors in irradiated apple cider. Apple cider irradiated in three plastic polymers, polystyrene (PS), low-density polyethylene (LDPE) or nylon-6 (N6), was compared to unirradiated cider packaged and stored in glass containers. Cider with (0.1%) and without (0%) potassium sorbate was evaluated initially following irradiation treatment and over a storage period of three weeks. Soluble solids, pH and titratable acidity measurements also help monitor changes caused by fermentation and other reactions which take place during storage. Volatile flavor compounds of apple cider were determined and quantified using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS).

Analytical Data

Significant interactions existed between packaging material and sorbate addition treatments for soluble solids content (SS), pH and titratable acidity (TA) of apple cider throughout Experiment I. Overall, soluble solids ranged between 11 and 12.8% (sucrose at 20°C) and decreased throughout three weeks of storage. pH values ranged from 3.81 to 4.09 during three weeks of storage but did not follow a clear trend. Titratable acidity (available acid content) increased during storage and ranged from 0.19 to 0.41g malic acid/100mL cider.

Natural yeasts present in apple products convert sugars into alcohol and organic acids during the initial stages of fermentation. During the final stages of fermentation, bacterial populations convert the alcohol into acetic acid, yielding apple cider vinegar (Stinson et al., 1979). Analytical measurements such as pH, titratable acidity and soluble solids content, therefore, can follow the steps which occur during fermentation. The results of this

experiment indicate a decrease in sugar content and an increase in acid during storage for certain treatments, which suggests the effects of a fermentation process.

Analysis of variance results showed significant interactions between packaging material, sorbate addition and storage time for soluble solids and titratable acidity content. These measurements were plotted versus time, therefore, in order to illustrate the effects of packaging material and sorbate addition during three weeks of storage (Figure 2 and 3).

When comparing the effect of sorbate for each treatment, soluble solids was significantly higher in the presence of sorbate for all treatments except cider packaged in LDPE (Figure 2a). In the absence of sorbate, unirradiated (raw) cider had significantly lower soluble solids content, decreasing from 12.3 to 10.5%, than all three irradiated samples following three weeks of refrigerated storage (Figure 2b). In the presence of sorbate, though, soluble solids contents were quite similar among unirradiated cider and irradiated cider in PS and N6, while cider irradiated in LDPE had a significantly lower soluble solids content.

Titratable acidity was significantly lower for samples with sorbate than it was for samples without sorbate among all four sample treatments. When comparing the four treatments, cider irradiated in LDPE without sorbate also had the highest titratable acidity following three weeks of storage (Figure 3b), increasing from 0.21 to 0.46 g malic acid/100mL cider, but the difference was not significant. In the presence of sorbate, differences between packaging materials were not evident (Figure 3a). These trends suggest the effect of sorbate in minimizing changes in soluble solids and acidity contents compared to cider without sorbate. It also suggests that in the absence of sorbate, LDPE is inferior to PS and N6 packaging materials in minimizing changes in soluble solids and acidity during storage.

The PS containers used in this experiment had virtually no oxygen permeability. N6 had a permeability of 2.6 cc-mil O_2/100 in^2/24 hr @ 25°C while LDPE had a permeability of 276.5 cc-mil O_2/100 in^2/24 hr @ 25°C. Since LDPE is inferior to PS and N6 in terms of controlling oxygen permeability, the process of fermentation was most likely escalated in LDPE film. This supports that LDPE film is inferior to the other two plastic polymers in deterring fermentation since soluble solids content was lowest and acid levels were highest in LDPE packaging following three weeks of storage.

Analytical data comparing the effects of preservative treatment supports the role which sorbate plays in limiting fermentation or other degradation reactions of irradiated and unirradiated apple cider. In the presence of 0.1% potassium sorbate, apple cider had higher soluble solids and lower titratable acidity contents following storage. Once again, this trend suggests the control of fermentation with less conversion of sugars into alcohol and acids, i.e. high soluble solids and low acidity. Similar findings reported by Luedtke and Powell (2002) and Baroody and McLellan (1986) suggests that sorbate increases shelf-life by inhibiting yeasts and molds in a low pH environment.

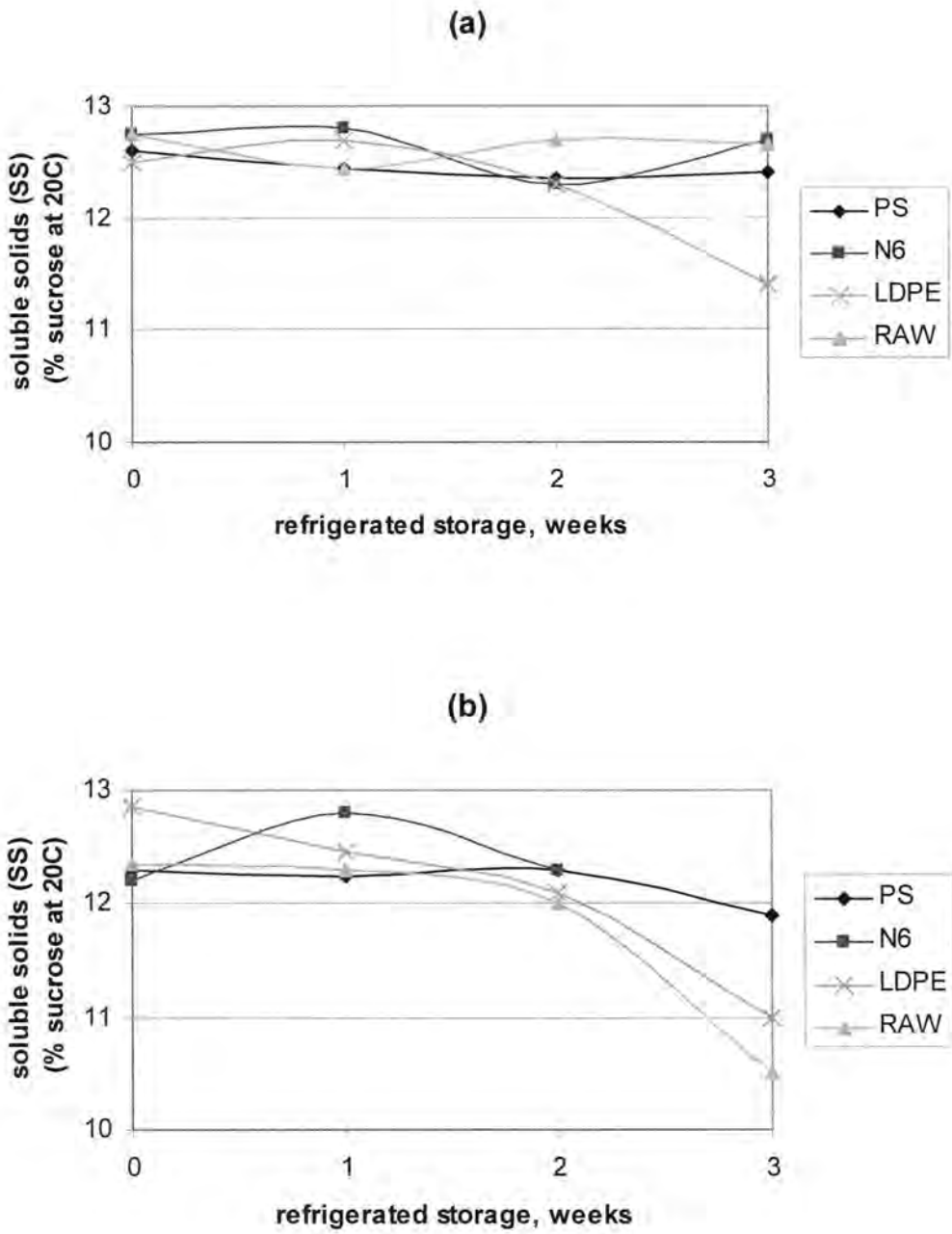


Figure 2. Effects of storage on soluble solids content of apple cider (a) with (0.1%) potassium sorbate and (b) without potassium sorbate using various packaging materials. Points represent means of two replications. PS = polystyrene containers (2kGy), N6 = nylon-6 polyamide pouches (2kGy), LDPE = low-density polyethylene pouches (2kGy), RAW = unirradated apple cider packaged in glass (0kGy).

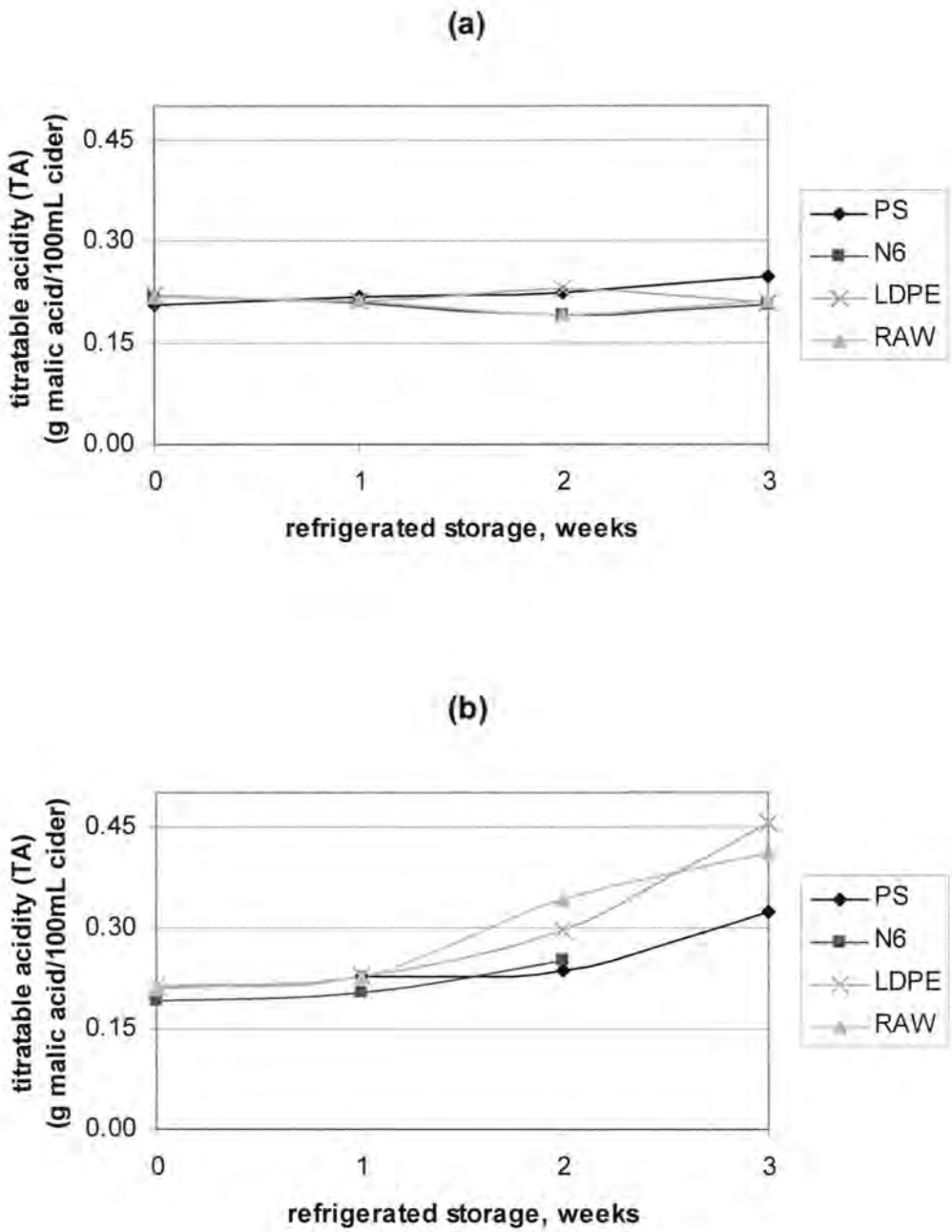


Figure 3. Effects of storage on titratable acidity content of apple cider (a) with (0.1%) potassium sorbate and (b) without potassium sorbate using various packaging materials. Points represent means of two replications. PS = polystyrene containers (2kGy), N6 = nylon-6 polyamide pouches (2kGy), LDPE = low-density polyethylene pouches (2kGy), RAW = unirradated apple cider packaged in glass (0kGy).

Volatile Flavor Analysis

Gas chromatography and GC-MS data obtained in the first experiment allowed for the identification and quantification of a significant number of volatile flavor compounds. Esters, especially acetates and butanoates, are known to be characteristic of apples and apple products (Flath et al., 1967; Dimick and Hoskin, 1983; Cunningham et al., 1986). Volatile ester compounds such as butyl acetate, 2-methyl butyl acetate, hexyl acetate, ethyl butanoate, ethyl-2-methyl butanoate, butyl butanoate, hexyl butanoate, ethyl hexanoate, and hexyl hexanoate were among the major compounds identified and quantified throughout this study.

Initial contents

In order to compare initial volatile flavor contents and the effects of irradiation treatment, analysis of variance was completed using GC peak areas from week 0. Table 1 lists the volatile flavor compounds and contents initially found in the four packaging treatments (raw and irradiated apple cider). Table 2 includes the initial contents of the volatile flavor compounds for which sorbate treatment was significant.

Table 1 suggests that a significant difference between packaging treatments existed only in propyl butanoate and phenylacetaldehyde contents. For propyl butanoate, LDPE had the lowest initial contents while phenylacetaldehyde had the lowest content for PS. In no other instance did unirradiated cider (glass) have significantly different initial contents of flavor compounds compared to cider that was irradiated in the three packaging materials. A direct effect of irradiation is not observed for a majority of the flavor compounds. It is important to mention that the threshold of these flavor compounds is unknown. Therefore, it is possible that propyl butyrate and phenylacetaldehyde do have an impact on characteristic

apple cider flavor. It is also possible that more variability exists in small peaks compared to larger peaks since standard deviations for compounds with undetected GC areas are lower.

The effect of sorbate addition on initial volatile flavor contents was significant for only two compounds, propyl acetate and butyl pentanoate. In the case of propyl acetate, cider without sorbate had a higher GC area. In the case of butyl pentanoate, however, cider with sorbate had a much higher initial GC area (Table 2). It is possible that sorbate had an effect on these flavor compounds in the first days following cider production. Since the producer added sorbate immediately following pressing, it was exposed to the cider for 2-3 days before the samples were packaged and irradiated. The effects of sorbate are expected to be even greater following storage. Interactions were between sorbate addition and packaging material were not significant.

Effects of storage

Initial statistical analysis as a 3-way factorial (packaging material, sorbate and storage week) for each flavor compound resulted in significant interactions between storage time and packaging material and/or sorbate addition. For this reason, gas chromatograph (GC) peak areas were plotted versus storage time in order to calculate slopes, i.e. the rate of loss in flavor intensity, for each principal flavor compound. As a result, a treatment variable was removed and the effects of only packaging material and sorbate remained.

Flavor compounds displayed the greatest decrease in intensity between week 0 and week 1 of storage and tended to start leveling off by week 3. Figure 4a shows the effect of storage on the content of hexyl acetate, a major characteristic apple flavor compound. A majority of the identified and quantified volatile flavor compounds show a similar storage effect. The rate of loss of flavor compounds is best described by first-order kinetics, as

shown by the plot of $\log_{10}$ GC peak area versus storage time (Figure 4b). The slope for the rate of loss for each flavor compound was calculated to determine the effects of packaging material and sorbate addition on the stability of the flavor compounds during storage.

Table 1. Effect of packaging materials on initial contents of apple cider flavor compounds[1]

	GC peak areas (week 0)			
Irradiation dose	0kGy	2kGy		
Packaging material[2]	glass	PS	N6	LDPE
ethyl propionate	31.0	43.7	32.1	65.7
propyl acetate[+]	65.5	42.5	39.8	61.5
t-butyl acetate	11.6	11.3	19.9	40.7
isobutyl acetate	6.6	8.3	10.6	10.6
methyl(ante)iso-pentanoate	22.2	10.4	10.9	83.3
hexanal	317.0	245.5	194.0	425.3
ethyl butanoate	491.0	380.7	273.0	480.4
1-methyl propyl acetate	54.1	55.3	61.5	70.4
butyl acetate	1869.7	1411.3	1694.5	1778.4
3-pentyl acetate	74.0	72.4	81.7	94.4
ethyl-2-methyl butanoate	1268.8	1238.3	1101.1	1263.0
c-3-hexen-1-ol	ND	ND	ND	ND
methyl-2-ethyl butanoate	ND	ND	31.4	ND
methyl-2-methyl pentanoate	125.8	52.8	54.2	22.3
hexanol	898.7	827.8	1010.1	1268.2
2-methyl butyl acetate	670.2	524.2	651.4	742.8
methyl iso-hexanoate	138.2	85.3	150.4	104.9
propyl butanoate	46.0 a	45.7 a	47.6 a	62.8 b
ethyl pentanoate	35.6	43.2	40.1	63.4
butyl propionate	64.2	96.8	67.8	88.1
pentyl acetate	135.8	204.6	154.4	144.5
methyl hexanoate	38.3	48.8	54.0	56.5
isopropyl 2-methyl butanoate	17.4	15.6	14.9	33.0
benzaldehyde	11.4	13.3	10.8	30.1
3-methyl butyl propionate	9.1	9.6	10.5	25.3
1-octene-3-ol	6.1	4.0	9.5	14.8
butyl butanoate	341.0	265.0	259.5	267.1
ethyl hexanoate	582.8	550.3	437.1	481.3
octanal	ND	ND	ND	ND

Table 1 (continued)[1]

	GC peak areas (week 0)			
Packaging material[2]	glass	PS	N6	LDPE
Irradiation dose	0kGy	2kGy	2kGy	2kGy
3-c-hexen-1-yl acetate	66.4	41.5	58.7	70.5
hexyl acetate	5450.8	4932.2	4152.3	4545.7
phenylacetaldehyde	6.5 b	ND a	13.6 b	17.5 b
butyl-2-methyl butanoate	80.0	68.0	56.0	66.8
t-2-octenal	28.3	18.1	22.0	37.5
1-octanol	152.5	112.3	136.1	177.3
butyl pentanoate[+]	139.0	85.5	142.1	132.2
propyl hexanoate	35.1	2.8	18.3	76.8
ethyl heptanoate	348.6	345.7	336.6	297.7
nonanal	78.6	60.5	60.9	90.8
hexyl propionate	92.0	ND	120.6	62.4
heptyl acetate	232.1	213.6	161.9	171.5
3-hepten-1-yl-acetate	332.2	ND	426.8	659.5
benzyl acetate	18.0	27.8	29.2	34.4
t-2-nonenal	106.4	85.6	98.5	125.7
hexyl butanoate	1525.9	1351.5	770.8	775.0
estragole	126.2	104.2	50.6	79.8
decanal	32.9	28.0	39.4	40.1
3-octen-1-yl acetate	25.9	23.2	16.6	21.3
hexyl-2-methyl butanoate	521.4	475.5	266.7	267.5
2-phenyl ethyl acetate	17.8	12.2	10.3	6.2
2-decenal	7.8	6.4	11.2	19.6
pentyl hexanoate	37.5	31.7	19.9	32.1
hexyl hexanoate	837.2	694.1	390.7	484.1
terpene ester	45.6	37.0	59.7	166.5
beta-farnesene	59.8	66.9	100.3	58.3
alpha-farnesene	1211.2	810.2	809.5	1029.1

[1]Compounds are presented in the order of elution from the gas chromatograph. Means are duplicate analyses of two replications (week 0) with data for sorbate addition pooled.
[2]glass = unirradiated apple cider, PS = polystyrene containers, N6 = nylon-6 polyamide pouches, LDPE = low-density polyethylene pouches.
[+]Effect of sorbate significant ($P<0.05$).

Table 2. Effect of sorbate on initial contents of propyl acetate and butyl pentanoate[1]

	GC peak areas (week 0)	
	0% sorbate	0.1% sorbate
propyl acetate[+]	70.8 b	33.8 a
butyl pentanoate[+]	49.4 a	200.0 b

[1]Means are duplicate analyses of two replications with data for packaging material pooled.
[+]Means with different letters (rows) demonstrate significantly sorbate effects ($P<0.05$).

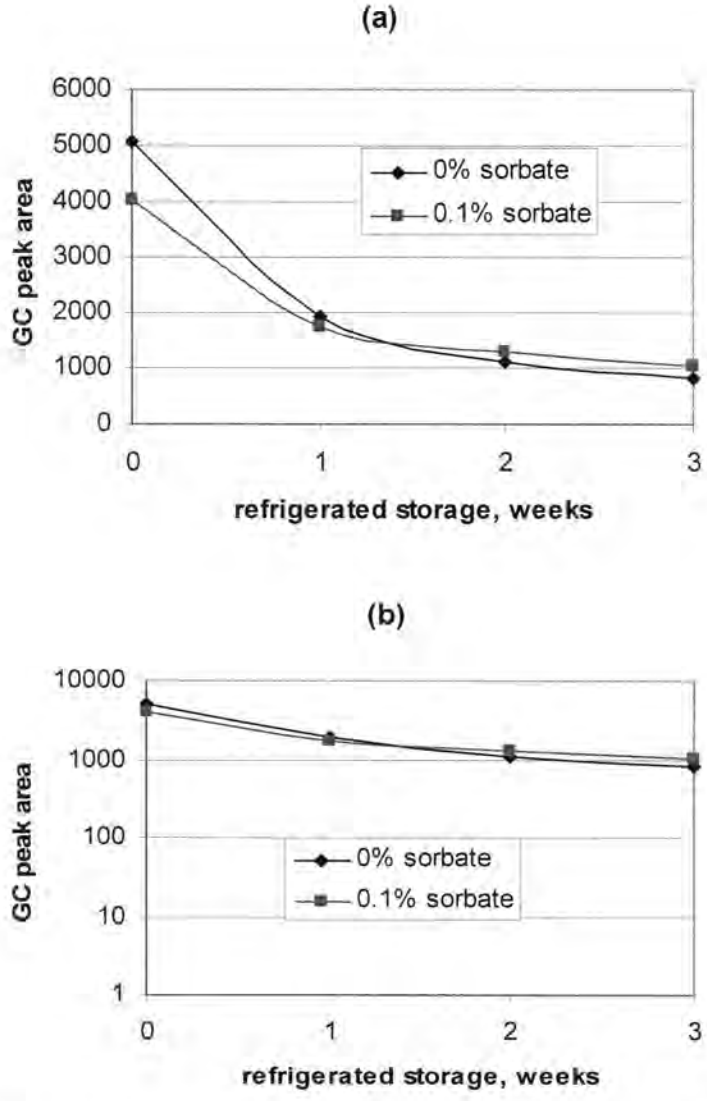


Figure 4. Effects of storage on content of hexyl acetate packaged in LDPE film using (a) linear GC peak area and (b) semi-$\log_{10}$ GC peak area plots versus storage time.

Principle Component Analysis

Principle Component Analysis (PCA) identifies patterns of interactions between variables to condense a large set of data into groups of similar characteristics. Since more than fifty volatile compounds were identified in the cider, PCA is more effective in demonstrating relationships between variables than correlation or other statistical techniques. In this experiment, linear slopes of analytical data (soluble solids, pH, and titratable acidity) and slopes of the log GC area vs. week were loaded onto the PCA function. The first four principle components (PC) accounted for more than 71% of the total variability in the data set. PC-1 (38.5%) contained soluble solids, titratable acidity and 27 volatile flavor compounds, PC-2 (12.5%) contained 7 flavor compounds, PC-3 (11.8%) contained 4 volatile flavor compounds and PC-4 (8.8%) contained 3 volatile flavor compounds (Figure 5).

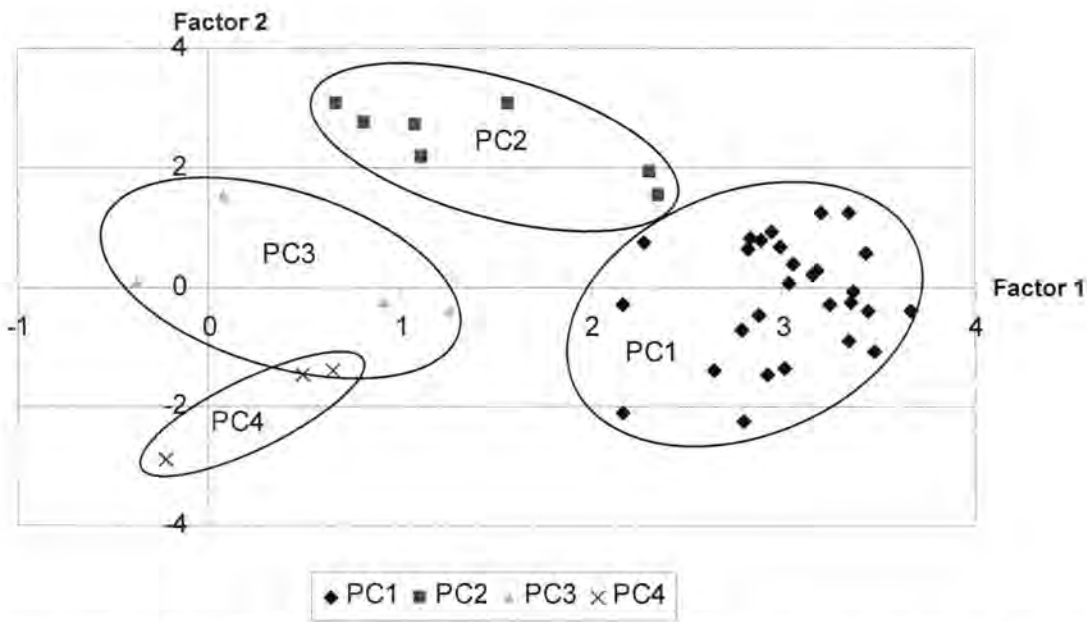


Figure 5. Plot of the PCA of apple cider showing associations between volatile flavor compounds and analytical data. Vector coordinates representing individual flavor compounds, soluble solids or titratable acidity signify pooled responses for all processing treatments (irradiation, packaging material and sorbate addition) using the rates of loss during three weeks of refrigerated storage for two replications.

Volatile flavor compounds were not exclusively grouped within the principal components based on the class of compound, i.e. alcohols, aldehydes, or esters. PC-1, however, did contain a majority of the characteristic esters known to contribute to apple flavor while PC-2 contained mostly alcohols. Furthermore, the classes of flavor compounds did not always respond similarly to the effects of packaging material or sorbate addition. The effects of packaging material and sorbate addition, therefore, will be discussed separately.

Effect of packaging material

A comparison of the slopes for each of the packaging materials illustrates the retention of flavor compounds in irradiated cider compared to untreated (raw) cider during three weeks of storage following irradiation treatment. Based on the data shown in Table 3, cider irradiated and stored in polystyrene (PS) containers had a statistically less negative slope (lower rate of loss) than untreated cider or irradiated cider packaged in low-density polyethylene (LDPE) for volatile flavor compounds characteristic to apple or fruity flavor and aroma. Examples of such trends can be noted for esters such as butyl acetate, 2-methyl butyl acetate, hexyl acetate, propyl butanoate, ethyl pentanoate, and t-2-octenal. In some cases, however, the raw, PS and nylon-6 (N6) were more similar to each other and LDPE had the most negative slopes overall. Such trends, which were identified in isopropyl 2-methyl butanoate, butyl propionate, butyl-2-methyl butanoate, t-2-nonenal and alpha-farnesene, suggest that LDPE demonstrates the poorest retention in characteristic apple flavors. Most of the compounds that displayed significant packaging effects loaded onto PC-1, which supports the grouping of characteristic apple cider flavor compounds together.

Willige et al. (2001) used flavor compounds to evaluate the absorption of different packaging materials. The extent of flavor absorption through packaging was influenced by

polymer polarity and flavor compound polarity. For example, plastic polymers have greater affinity for compounds of a similar polarity than those of dissimilar polarity. Polar flavor compounds are attracted to more polar packaging materials to a larger degree than less-polar flavor compounds. This is one factor that can explain packaging effects.

A study by Ayhan et al. (2001) found that packaging materials affects the retention of orange juice aroma compounds. Four packaging materials (glass, polyethylene terephthalate (PET), high-density polyethylene (HDPE) and low-density polyethylene (LDPE)) were evaluated. Retention of all flavor compounds in the juice was found to be significantly higher in glass and PET than in HDPE and LDPE. More specifically, the loss of aldehydes and ethyl butanoate was highest in HDPE and LDPE bottles. According to Matsui et al. (1992), LDPE has inferior gas barrier properties, and the sorption of flavor compounds is more distinct in LDPE than in other films. The data shown in Table 3 suggests that LDPE was the poorest in retaining characteristic apple cider flavors while PS was the best.

The raw cider which was stored in glass jars had a negative slope even though volatile flavors can not permeate through glass. Since glass is known to be inert in terms of flavor absorption, loss of flavor compounds in glass bottles is thought to be related to chemical degradation (Ayhan et al., 2001). Since the cider which was stored in glass bottles was not irradiated, it is speculated that microorganism populations were much higher and the opportunity for fermentation and other degradation reactions was great.

Table 3. Effects of packaging treatments on the retention of analytical data and volatile flavor compounds of apple cider during three weeks of refrigerated storage[1]

	SLOPE (log GC peak area/week)							
Packaging material[2]	glass		PS		N6		LDPE	
Irradiation dose	0kGy		2kGy		2kGy		2kGy	
COMPONENT 1 (PC-1)								
SS[3]	-0.295	b	-0.092	c	-0.012	c	-0.480	a
TA[4]	0.034	b	0.031	b	0.007	a	0.039	b
1-methyl propyl acetate	-0.111		-0.040		0.065		-0.133	
butyl acetate	-0.092	a	-0.011	b	-0.069	a	-0.100	a
2-methyl butyl acetate	-0.050	a	0.018	b	-0.029	ab	-0.073	a
pentyl acetate	-0.116		-0.077		-0.135		-0.170	
hexyl acetate	-0.148	ab	-0.057	c	-0.094	bc	-0.227	a
heptyl acetate	-0.139		-0.086		-0.044		-0.160	
benzyl acetate	-0.006		-0.020		-0.048		-0.112	
butyl propionate*	-0.124	b	-0.041	b	-0.066	b	-0.216	a
ethyl-2-methyl butanoate	-0.213		-0.097		-0.103		-0.174	
propyl butanoate	-0.071	b	0.027	c	-0.050	b	-0.156	a
isopropyl 2-methylbutanoate	-0.036	b	0.018	b	-0.014	b	-0.339	a
butyl butanoate	-0.101	ab	-0.009	b	-0.037	b	-0.163	a
butyl-2-methyl butanoate	-0.030	b	0.036	b	0.023	b	-0.142	a
hexyl butanoate	-0.325		-0.131		0.064		-0.299	
hexyl-2-methyl butanoate	-0.063		-0.033		0.142		-0.284	
ethyl pentanoate	-0.041	b	0.056	c	-0.035	b	-0.127	a
methyl(ante)iso-pentanoate	-0.099		0.100		0.167		-0.105	
methyl hexanoate	-0.181		-0.092		-0.139		-0.274	
ethyl hexanoate	-0.149		-0.076		-0.067		-0.212	
pentyl hexanoate	-0.323	a	-0.129	ab	0.022	b	-0.300	a
ethyl heptanoate	-0.308		-0.080		-0.143		-0.352	
propyl hexanoate	0.339		0.264		-0.099		0.139	
hexanal	-0.607		-0.365		-0.323		-0.415	
t-2-octenal	-0.253	a	0.005	b	-0.022	b	-0.308	a
decanal	-0.024		0.005		-0.019		-0.080	
benzaldehyde	-0.391		-0.121		-0.092		-0.247	
alpha-farnesene	-0.111	b	-0.061	b	-0.040	b	-0.341	a
COMPONENT 2 (PC-2)								
3-pentyl acetate	-0.640		-0.464		-0.673		-0.516	
3-methylbutyl propionate	-0.009		0.025		-0.057		-0.067	
1-hexanol	-0.018		0.028		-0.045		-0.027	
1-octene-3-ol	-0.113		0.029		-0.257		-0.139	
1-octanol	-0.066		0.043		-0.020		-0.043	
t-2-nonenal	0.078	b	0.090	b	0.045	b	-0.013	a
terpene ester	-0.103		-0.016		-0.177		-0.103	

Table 3 (continued)[1]

Packaging material[2] / Irradiation dose	SLOPE (log GC peak area/week) glass 0kGy	PS 2kGy	N6 2kGy	LDPE 2kGy
COMPONENT 3 (PC-3)				
methyl iso-hexanoate	-0.310	-0.615	-0.169	0.300
octanal	ND	ND	ND	0.157
estragole	-0.325	-0.131	0.064	-0.299
beta-farnesene	-0.015	-0.035	-0.105	-0.180
COMPONENT 4 (PC-4)				
isobutyl acetate	-0.103	0.037	-0.037	0.044
2-phenyl ethyl acetate	-0.091	-0.070	0.300	0.146
3-octen-1-yl acetate	-0.063	0.037	0.020	0.036

[1]Means are duplicate analyses of two replications with data for sorbate addition pooled. Means followed by different letters within the same row are significantly different from each other ($P<0.05$).
[2]glass = unirradiated apple cider, PS = polystyrene containers, N6 = nylon-6 polyamide pouches, LDPE = low-density polyethylene pouches.
[3]SS = soluble solids (% sucrose at 20°C). Slope = decrease in SS/week in storage.
[4]TA = titratable acidity (g malic acid/100mL cider). Slope = decrease in TA/week in storage.
*butyl propionate slopes display a significant interaction between packaging material and sorbate addition.

Effect of sorbate addition

The addition of 0.1% potassium sorbate had a beneficial effect in slowing the loss of flavor compounds during storage compared to cider without sorbate. In all cases where a significant difference was noted between the presence and absence of preservative, rates of loss were greater (more negative slope) in cider that did not contain sorbate (Table 4). Soluble solids decreased and titratable acidity increased at a lower rate in the presence of sorbate than when sorbate was absent. All of the volatile flavor compounds that showed a significant effect of sorbate addition loaded onto PC-1 and were characteristic apple cider esters. Without the addition of preservative, therefore, characteristic volatile flavor compounds are not retained as well during three weeks of storage as cider with preservative.

Fermentation or other degradation reactions most likely diminish the apple or fruity compounds that give apple cider desirable quality.

According to Thaker and Arya (1993), orange juice and mango pulp decreased in characteristic fruit aroma as a result of irradiation (10kGy). The addition of sorbate, however, "completely prevented the formation of irradiation induced off-flavors" (Thaker and Arya, 1993). Another study reported that the effects of processing treatment (irradiation and pasteurization) was related to the presence of sorbate. According to Boylston et al. (2003), the presence of 0.1% sorbate reduced the effects of irradiation (i.e. a decrease in characteristic apple cider esters) on volatile flavor compounds. It is thought that sorbic acid acts as a scavenger of hydrogen and hydroxyl radicals which are produced during irradiation. If natural antioxidants such as ascorbic acid are present in a fruit juice, they are not considerably reduced if potassium sorbate is present during irradiation. It should be noted that ascorbic acid is present in apples, but the process of cider pressing causes complete loss of ascorbic acid by the action of polyphenol oxidase. Only if ascorbic acid is re-added to apple cider following pressing is it present in the final product.

Interactions of Packaging Material and Sorbate Addition

The interaction of package type and preservative content for slope values was observed for one of the volatile compounds, butyl propionate (Table 5). With the addition of 0.1% sorbate, differences between packaging treatments were not significant. Without the presence of sorbate, however, PS and N6 means had significantly lower rates of loss than glass and LDPE treatments. Potassium sorbate, therefore, did cause some interactions in the rate of loss for butyl propionate.

Table 4. Effects of sorbate addition on the retention of analytical data and volatile flavor compounds of apple cider during three weeks of refrigerated storage[1]

	SLOPE (log GC peak area/week)			
	0% sorbate		0.1% sorbate	
COMPONENT 1 (PC-1)				
SS[2]	-0.361	a	-0.122	b
TA[3]	0.061	b	0.001	a
1-methyl propyl acetate	-0.119		0.009	
butyl acetate	-0.116	a	-0.020	b
2-methyl butyl acetate	-0.062	a	-0.005	b
pentyl acetate	-0.189	a	-0.060	b
hexyl acetate	-0.164	a	-0.098	b
heptyl acetate	-0.242	a	0.028	b
benzyl acetate	-0.079		-0.014	
butyl propionate*	-0.176	a	-0.047	b
ethyl-2-methyl butanoate	-0.250	a	-0.044	b
propyl butanoate	-0.097	a	-0.028	b
isopropyl 2-methyl butanoate	-0.140	a	-0.046	b
butyl butanoate	-0.151	a	-0.004	b
butyl-2-methyl butanoate	-0.053		-0.004	
hexyl butanoate	-0.288		-0.058	
hexyl-2-methyl butanoate	-0.078		-0.041	
ethyl pentanoate	-0.076	a	0.003	b
methyl(ante)iso-pentanoate	-0.024		0.055	
methyl hexanoate	-0.254	a	-0.089	b
ethyl hexanoate	-0.200	a	-0.052	b
pentyl hexanoate	-0.260		-0.105	
ethyl heptanoate	-0.430	a	-0.012	b
propyl hexanoate	0.370	a	-0.144	b
hexanal	-0.641		-0.214	
t-2-octenal	-0.287	a	-0.002	b
decanal	-0.065		0.006	
benzaldehyde	-0.292		-0.133	
alpha-farnesene	-0.191	a	-0.086	b
COMPONENT 2 (PC-2)				
3-pentyl acetate	-0.668		-0.478	
3-methyl butyl propionate	-0.022		-0.032	
1-hexanol	-0.019		-0.012	
1-octene-3-ol	-0.151		-0.089	
1-octanol	-0.033		-0.010	
t-2-nonenal	0.043		0.057	
terpene ester	-0.120		-0.080	

Table 4 (continued)[1]

	SLOPE (log GC peak area/week)	
	0% sorbate	0.1% sorbate
COMPONENT 3 (PC-3)		
methyl iso-hexanoate	-0.235	-0.097
octanal	-0.011	0.326
estragole	-0.288	-0.058
beta-farnesene	-0.083	-0.084
COMPONENT 4 (PC-4)		
isobutyl acetate	-0.047	0.017
2-phenyl ethyl acetate	0.138	-0.015
3-octen-1-yl acetate	0.018	-0.003

[1]Means are duplicate analyses of two replications with data for packaging material pooled. Means followed by different letters within the same row are significantly different from each other ($P<0.05$).
[2]SS = soluble solids (% sucrose at 20°C).
[3]TA = titratable acidity (g malic acid/100mL cider).
*Compound slope displays a significant interaction between packaging and sorbate.

Table 5. Interaction of packaging material and sorbate addition for butyl propionate[1]

Packaging material[2] (irradiation dose)	SLOPE (log GC peak area/week) 0% sorbate	0.1% sorbate
Glass (0kGy)	-0.246 ax	-0.003 bx
PS (2kGy)	-0.026 ay	-0.055 ax
N6 (2kGy)	-0.122 ay	-0.009 ax
LDPE (2kGy)	-0.311 ax	-0.120 bx

[1]Means are duplicate analyses of two replications. Means followed by different letters within the same row are significantly different among sorbate addition ($P<0.05$; a,b). Means followed by different letters within the same column are significantly different among packaging treatment ($P<0.05$; x,y).
[2]PS = polystyrene, N6 = nylon-6 pouches, LDPE = low-density polyethylene pouches.

Increasing Content of Nonanal during Storage

One compound, nonanal, increased in the presence of preservative as a function of storage time. In this case, linear regression of GC peak area versus week made a better correlation of the relationship. Over three weeks in storage, cider with sorbate showed nonanal to have a positive slope based on GC peak area (Table 6). Since irradiation causes the formation of free radicals and sorbic acid scavenges these radicals (Thaker and Arya, 1993), it is speculated that nonanal is a breakdown product of irradiated sorbate. This would support a trend that nonanal increases when 0.1% sorbate is present in an irradiated sample. Research completed by Marth et al. (1996) suggests that decarboxylation of sorbic acid results in the formation of 1,3-pentadiene in feta cheese. It is also possible that 1,3-pentadiene contributes to off-flavor in response to the presence of sorbate. This compound could be present in low levels which are undetectable by gas-chromatography.

Table 6. Regression slopes of nonanal as a linear function of GC peak area versus week[1]

	SLOPE (GC peak area/week)			
	0% sorbate		0.1% sorbate	
nonanal	-7.110	a	17.964	b

[1]Means are duplicate analyses of two replications with data for packaging material pooled. Means followed by different letters within the same row are significantly different from each other ($P<0.05$).

Conclusions of Experiment I

Based on the data from Experiment I, the effects of packaging material and sorbate addition are important for retaining characteristic apple cider flavor and quality attributes. Cider irradiated and stored in PS and N6 packaging materials had lower rates of volatile flavor loss than untreated cider (in glass) or irradiated cider packaged in LDPE. In addition,

cider with 0.1% potassium sorbate had lower rates of volatile flavor loss than cider without sorbate. Potassium sorbate also tended to limit the decrease in soluble solids content and increase in acidity which takes places as a result of natural fermentation processes. Even though glass does not allow the permeation or absorption of gases or flavors, apple cider packaged in this material showed a loss of characteristic flavor as a result of natural fermentation processes and chemical reactions. The process of irradiation, therefore, is quite important to restrict deteriorative processes yet maintain characteristic apple cider flavor following treatment.

Based on the results of Experiment I, packaging materials affect the retention of characteristic apple cider flavor compounds. Since the apple cider evaluated in Experiment I was exposed to atmospheric air conditions, it is unknown whether the gas environment of irradiated apple cider affects analytical data (soluble solids, pH, titratable acidity), the retention of volatile flavor compounds, or microorganism populations. Experiment II, therefore, was completed in order to expose apple cider to three gaseous environments prior to irradiation and investigate the quality changes which take place during seven weeks of refrigerated storage.

Experiment II: Effect of gaseous environment and sorbate addition to maintain flavor characteristics of irradiated apple cider during storage

Flavor loss during storage is caused by chemical degradation of flavor components and development of off-flavors caused by such degradation. Sizer et al. (1988) explained how oxygen, utilized during decomposition reactions, can (1) be dissolved into a product, (2) diffuse through packaging material, or (3) dissolve into the product from package headspace.

Sizer et al. (1988) noted the importance of packaging orange juice in an inert environment in order to avoid the detrimental effects of oxygen on the retention of flavor, vitamin C and color. The presence of oxygen dissolved in apple cider or in the sample headspace may be important to the quality attributes of apple cider during irradiation and storage.

The objectives of Experiment II were to determine the effects of gaseous environment and sorbate addition on apple cider flavor during seven weeks of storage. Unirradiated (raw) apple cider was packaged in polystyrene (PS) containers and compared to cider irradiated and stored in PS under three gaseous environments, atmospheric air, nitrogen-flush and oxygen-flush. Cider with (0.1%) and without (0%) potassium sorbate was evaluated initially following treatment and over a storage period of seven weeks. Soluble solids, pH, titratable acidity and microbiological measurements were also determined to monitor quality changes which take place during storage. Volatile flavor compounds of apple cider were determined and quantified using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS).

Analytical Data

Soluble solids content (SS), pH and titratable acidity (TA) of apple cider varied throughout Experiment II. Since the two replications of Experiment II came from different cider processing dates, it is expected that more variation would exist in Experiment II than in Experiment I. Differences in the cultivar blend of apples used for production and apples that differ by degree of maturity and storage between two processing weeks can affect the initial contents of analytical data and volatile flavor profiles.

Analysis of variance results showed significant interactions between gas environment, sorbate addition and/or storage time for soluble solids and titratable acidity content. These

measurements were plotted versus time to illustrate the effects of gaseous environment and sorbate addition during seven weeks of storage (Figure 6 and 7). Soluble solids ranged between 10 and 14%, decreasing throughout seven weeks of storage in the absence of sorbate (Figure 6b). pH values ranged between 3.41 and 3.66 during seven weeks of storage (data not shown) and titratable acidity ranged between 0.33 to 0.75g malic acid/100mL cider and increased during storage in the absence of sorbate (Figure 7b).

The effect of sorbate on soluble solids content was significant among all four sample treatments. Unirradiated (raw) cider without sorbate had significantly lower soluble solids following seven weeks of refrigerated storage than all three irradiated ciders which were exposed to different gas environments (Figure 6b). This trend was also noted in Experiment I. In the presence of sorbate, though, soluble solids contents were quite similar among all four sample treatments and no significant differences existed (Figure 6a). A noticeable decrease in soluble solids took place after week 3 of storage in cider without sorbate. When sorbate is present, soluble solids actually increased slightly from the previous weeks. It should also be noted that overall soluble solids were higher in cider with sorbate. This is expected since sorbate provides additional solids and slows the effects of fermentation.

Titratable acidity measurements followed similar statistical trends during storage as soluble solids content but acidity increased with time (Figure 7). Raw cider without sorbate had significantly higher titratable acidity readings than all irradiated samples following seven weeks of storage, increasing from 0.34 to 0.70 g malic acid/100 mL cider (Figure 7b). A considerable increase in acidity began to occur after week 2 of storage. Though irradiated samples without sorbate were not statistically different, nitrogen-flush cider yielded the next highest acidity, followed by atmospheric air, while oxygen-flush samples had the lowest

acidity measurements following storage. An abundance of oxygen limits anaerobic metabolism and fermentation processes compared to cider from a nitrogen-flush environment. Yeast populations are expected to decline or remain steady in high-oxygen environments since yeasts prefer an anaerobic environment for growth and sugar metabolism (Jay, 2000). In the presence of sorbate, differences between gas environments were not significant (Figure 7a). Raw cider did have the highest final acidity content but the three irradiated cider treatments with sorbate did not differ greatly following storage.

Microbiological analyses based on yeast and aerobic plate counts are helpful in tracking the growth of microorganisms throughout seven weeks of storage. Initial yeast and aerobic bacteria counts (week 0) showed that raw cider had up to three $\log_{10}$ higher magnitudes of microorganisms compared to irradiated samples. Although analysis of variance did not find any statistical differences for aerobic or yeast counts among sample treatments, plots of the data during storage do show noticeable affects of sorbate addition to minimize the growth of microorganisms compared to cider without sorbate.

Yeast levels were sporadic throughout storage for samples with sorbate, while samples without sorbate followed an increasing trend after week 1 (Figure 8). The effect of sorbate was quite apparent when comparing yeast counts among sample treatments. In the absence of sorbate, yeast levels reached higher than 10^6, even for irradiated samples. In the presence of sorbate, however, yeast counts remained below 10^4 in irradiated apple cider and below 10^5 for raw cider. These trends illustrate the importance of sorbate in limiting the effects of fermentation when yeast counts are compared.

Aerobic plate counts did not follow clear trends like the yeast data. In the presence of sorbate, however, raw cider displayed aerobic counts much higher than cider which was

irradiated (Figure 9a). In the absence of sorbate, irradiated cider samples had aerobic counts which escalated after week 1 (Figure 9b). By week 7, irradiated cider had aerobic counts near that of raw cider and oxygen-flushed cider aerobic counts surpassed all other samples. This is expected since the bacteria in the oxygen-flush environment had more oxygen available in their environment.

The number of microorganisms present in a food is not the only determining factor for product spoilage. Initial levels and types of microorganisms present, or those present which can adapt to storage conditions, are more important in affecting shelf-life (Murdock and Hatcher, 1975). Many yeasts and bacteria which are naturally present in apple cider may be resistant to the irradiation process and presence of sorbate. According to Bills et al. (1982) the yeast strain *Saccharomyces rouxi*, when preconditioned in 0.1% sorbate, is tolerant to high sugar and sorbate solutions at specific pH levels. A separate study reported that sensitivity to sorbate is species dependent and organic acids can improve the antimicrobial action of potassium sorbate (Restaino et al., 1981).

Overall, analytical data supported trends of decreasing soluble solids and increasing titratable acidity, especially in cider without sorbate. In the presence of sorbate, storage effects were similar for soluble solids and acidity contents among irradiated and unirradiated cider exposed to the three gaseous environments. The importance of sorbate was also seen in yeast and aerobic bacteria data. Yeast counts for irradiated cider without sorbate (all three gas environments) increased to the same levels of unirradiated cider by week 7. Sorbate minimized increases in aerobic bacteria counts for irradiated cider compared to raw cider.

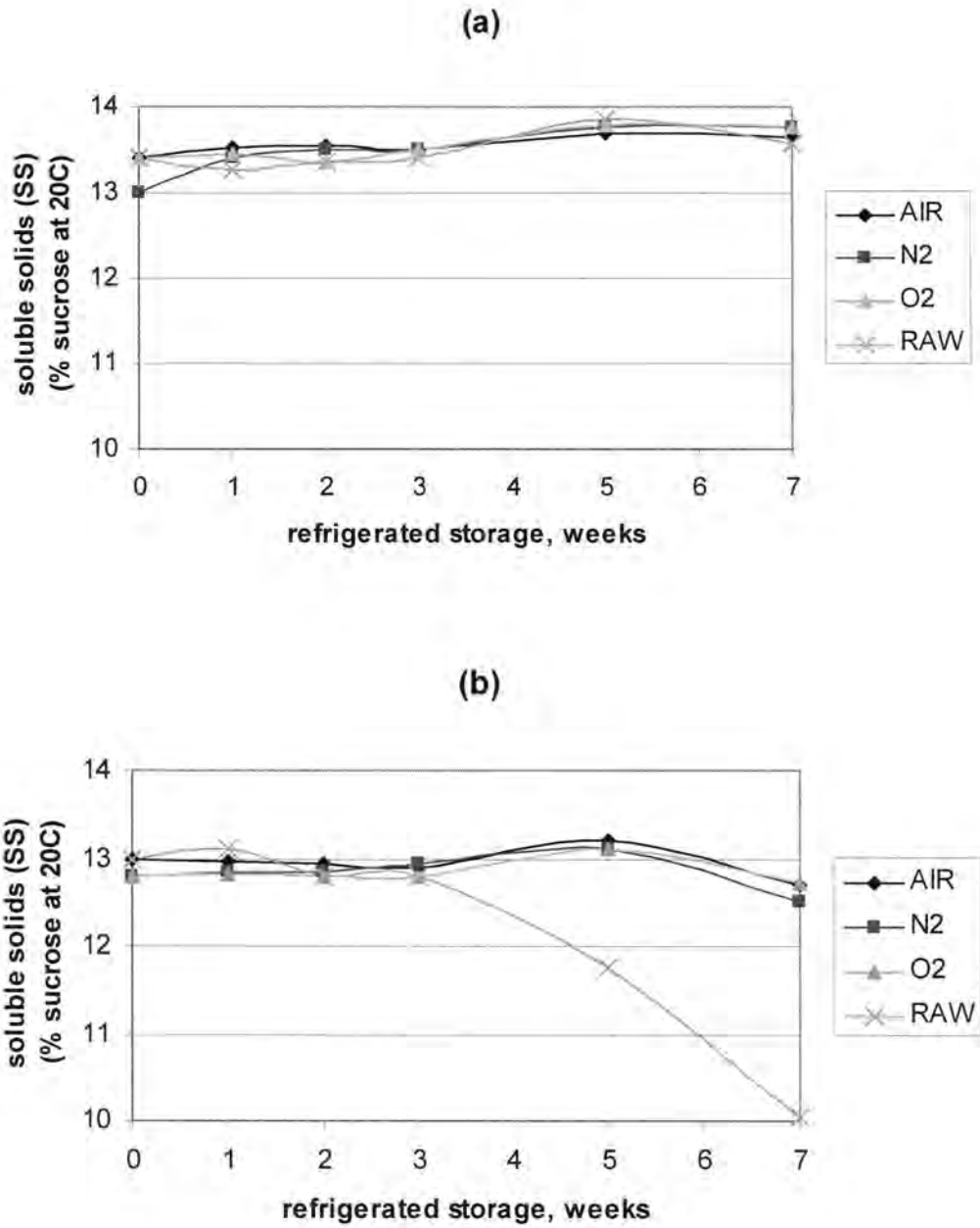


Figure 6. Effects of storage on soluble solids content of apple cider (a) with (0.1%) potassium sorbate and (b) without potassium sorbate using various gaseous environments. Points represent means of two replications. AIR = atmospheric air exposure (2kGy), N2 = nitrogen-flush exposure (2kGy), O2 = oxygen-flush exposure (2kGy), RAW = unirradated apple cider packaged in glass (0kGy), atmospheric air exposure.

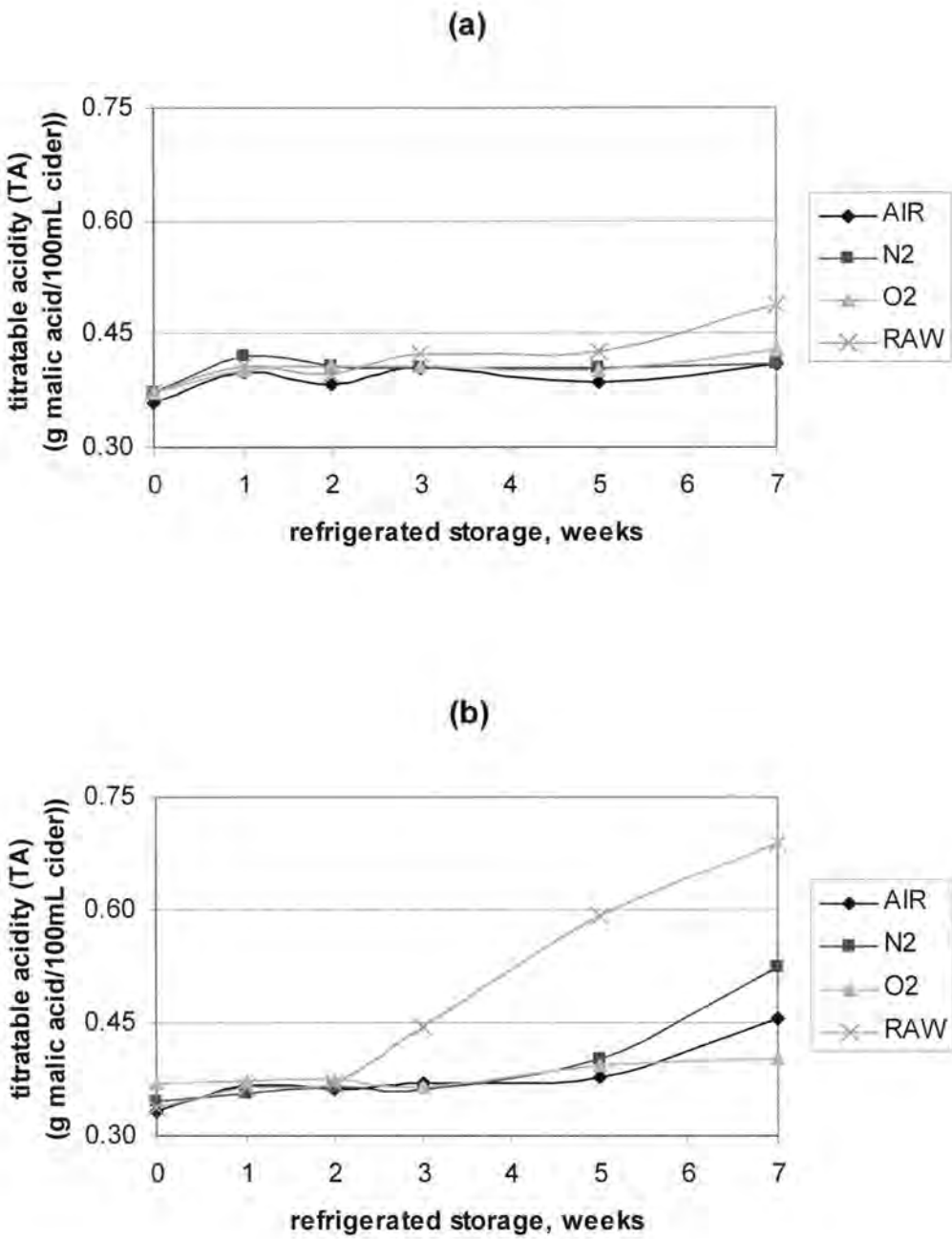


Figure 7. Effects of storage on titratable acidity content of apple cider (a) with (0.1%) potassium sorbate and (b) without potassium sorbate using various gaseous environments. Points represent means of two replications. AIR = atmospheric air exposure (2kGy), N2 = nitrogen-flush exposure (2kGy), O2 = oxygen-flush exposure (2kGy), RAW = unirradated apple cider packaged in glass (0kGy), atmospheric air exposure.

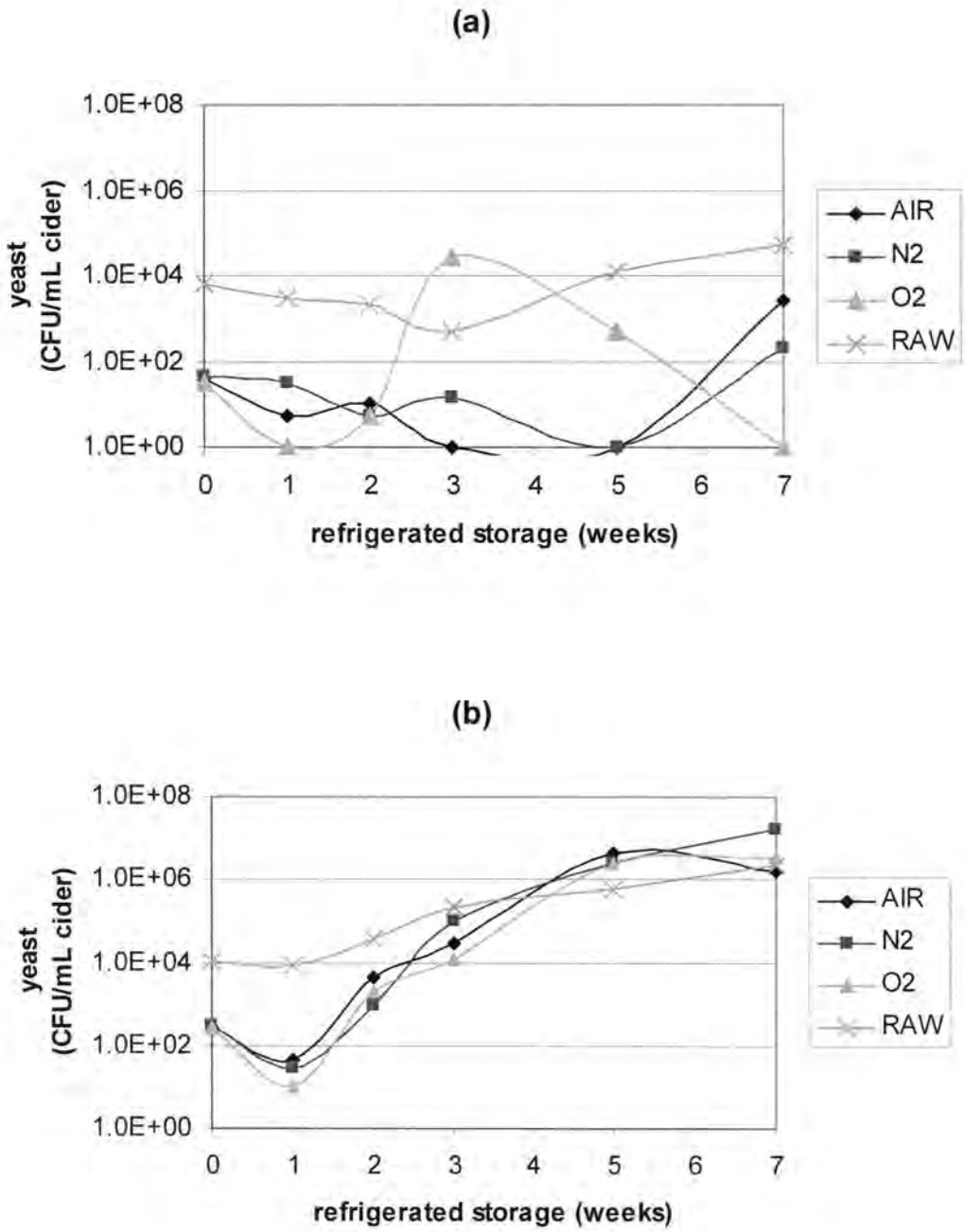


Figure 8. Effects of storage on yeast counts of apple cider (a) with (0.1%) potassium sorbate and (b) without potassium sorbate using various gaseous environments. Points represent means of two replications. AIR = atmospheric air exposure (2kGy), N2 = nitrogen-flush exposure (2kGy), O2 = oxygen-flush exposure (2kGy), RAW = unirradated apple cider packaged in glass (0kGy), atmospheric air exposure.

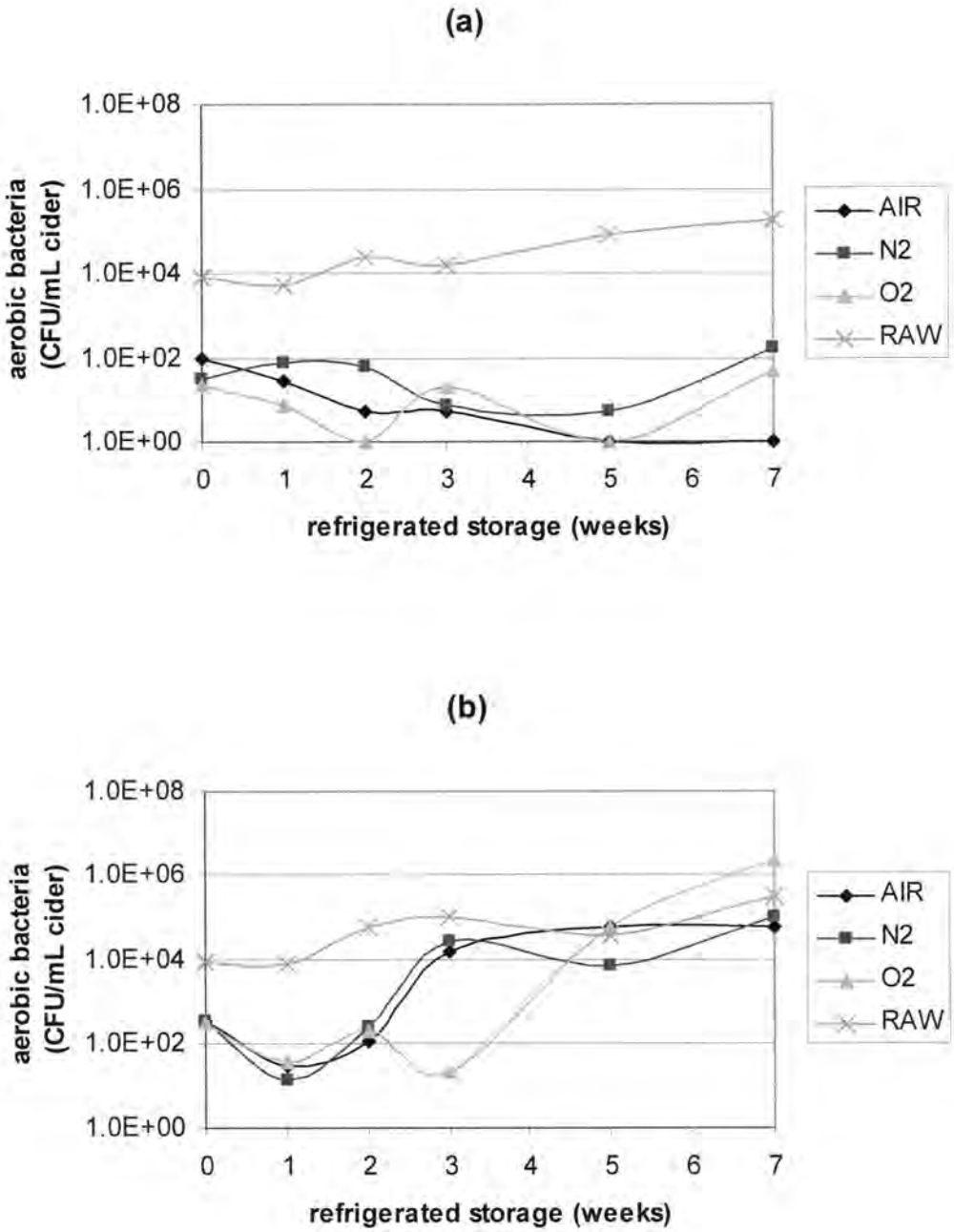


Figure 9. Effects of storage on aerobic plate counts of apple cider (a) with (0.1%) potassium sorbate and (b) without potassium sorbate using various gaseous environments. Points represent means of two replications. AIR = atmospheric air exposure (2kGy), N2 = nitrogen-flush exposure (2kGy), O2 = oxygen-flush exposure (2kGy), RAW = unirradated apple cider packaged in glass (0kGy), atmospheric air exposure.

Volatile Flavor Analysis

Gas chromatography and GC-MS data from Experiment II yielded most of the same volatile flavor compounds as in Experiment I that are known to be characteristic of apple cider. The most predominant ester compounds, such as butyl acetate, 2-methyl butyl acetate, hexyl acetate, ethyl butanoate, ethyl-2-methyl butanoate, butyl butanoate, hexyl butanoate, ethyl hexanoate, and hexyl hexanoate, were again identified and quantified in the second experiment.

Initial contents

Table 7 lists the initial contents of the identified volatile flavor compounds found in all four sample treatments following irradiation but prior to storage. Significant differences in initial GC peak areas existed for only four of the forty-four identified flavor compounds. In the case of butanol, hexanol, and decanal, an oxygen-flush (O_2) environment yielded higher GC peak areas while nitrogen-flush had lower areas for week 0 data (Table 7). For ethyl-2-methyl butanoate, N_2 cider had the lowest areas while raw cider had the highest.

The above findings may suggest that the process of irradiation causes a decrease in concentration of specific apple cider flavor compounds. Since the thresholds or contribution of these compounds to characteristic apple cider flavor is unknown, it can not be concluded whether the effects of irradiation were significant in terms of overall flavor profiles. If a compound has a low threshold, it can have a significant impact on the flavor of a food. For a majority of the flavor compounds, though, initial contents were not significant as a result of irradiation. For the compounds which were significant, it seems that oxidation plays a role in differences since oxygen-flush cider had higher GC peak areas compared to nitrogen-flush cider. Based on an orange juice study, an increase in volatile compounds in irradiated

samples may be due to the release of compounds from pectin structure and/or the decomposition of orange oils (Foley et al., 2002). Apple cider may also release volatile compounds from pectin as a result of irradiation.

The effect of sorbate was significant in initial contents of seven volatile flavor compounds (Table 8). In all of these compounds, cider with sorbate yielded higher GC peak areas than cider without sorbate. This shows the impact that potassium sorbate can play in preserving characteristic apple cider in the first days following production. These results support the findings of Deol (2003) that apple cider volatile flavor output increases in the presence of sorbate.

The interaction between gas environment and sorbate addition was significant in the initial contents of butanol (Table 9). In the absence of sorbate, no significant differences existed among gas environments for this compound. In the presence of sorbate, however, oxygen-flush cider had a much higher GC peak area than all three treatments. When yeast counts are considered in Figure 8, yeast populations are not significantly higher for oxygen-flush samples. It is possible, then, that butanol increases as a chemical reaction in response to the presence of potassium sorbate instead of a fermentation process.

Table 7. Effect of gas environment on initial GC peak areas of apple cider flavor compounds[1]

	GC peak areas (week 0)							
Gas environment[2]	AIR		N2		O2		RAW	
methanol	ND		ND		ND		ND	
ethanol	8.3		11.2		27.3		ND	
propanol (RI 0689)*	49.4		10.6		54.0		13.3	
ethyl propionate (RI 0704)*	187.3		148.6		232.9		140.2	
ethyl-2-methyl propionate (RI 0756)*	7.2		11.3		13.0		11.0	
methyl-2-methyl butanoate (RI 0776)*	72.7		32.4		48.3		30.5	
butanol[+] (RI 0797)*	76.9	b	50.5	b	155.2	c	26.8	a
hexanal	354.2		425.3		517.8		315.9	
ethyl butanoate	163.9		44.8		61.6		179.3	
1-methyl propyl acetate	60.2		56.6		66.8		61.1	
butyl acetate	2010.6		2267.4		2471.1		2103.0	
3-pentyl acetate	121.3		114.0		140.0		133.9	
ethyl-2-methyl butanoate	3568.9	ab	3116.8	a	4547.0	bc	4660.2	c
2-hexenal	176.7		154.3		216.9		217.1	
hexanol	839.4	ab	769.6	a	990.1	c	926.9	bc
2-methyl butyl acetate	1438.5		1560.2		1625.8		1617.3	
propyl butanoate	308.2		322.7		319.9		347.0	
butyl propionate	121.2		119.6		137.9		114.7	
pentyl acetate	273.3		277.9		340.3		323.6	
isopropyl-2-methyl butanoate	19.9		21.7		21.6		33.2	
benzaldehyde	27.9		27.3		34.9		28.3	
1-octen-3-ol	14.5		16.5		16.5		15.8	
butyl butanoate	447.0		446.3		496.3		472.1	
ethyl hexanoate	73.8		71.3		80.3		87.8	
hexyl acetate	9031.4		9078.7		9808.2		9731.2	
butyl-2-methyl butanoate	231.0		236.8		243.4		246.5	
pentyl butanoate	22.6		24.3		26.5		20.8	
1-octanol	ND		ND		ND		ND	
propyl hexanoate[+]	282.9		262.3		256.4		272.5	
nonanal[+]	124.6		160.7		143.1		139.5	
hexyl propionate	239.1		361.6		242.2		251.3	
heptyl acetate[+]	139.0		128.2		147.0		84.2	
hexyl-2-methyl propionate	10.4		27.5		1029.5		1026.7	
RI 1136*	33.1		26.2		30.8		35.0	

Table 7 (continued)[1]

Gas environment[2]	GC peak areas (week 0) AIR		N2		O2		RAW	
benzyl acetate	17.2		7.9		25.3		25.0	
t-2-nonenal	19.3		16.0		16.3		17.0	
hexyl butanoate[+]	1234.6		1202.4		1267.2		1153.6	
p-allyl anisole	108.2		105.8		112.9		97.2	
decanal	23.8	b	7.7	a	42.9	c	6.3	a
hexyl-2-methyl butanoate	655.5		649.2		696.7		612.2	
2-decenal	113.1		106.8		128.9		99.3	
1-decanol	12.6		9.4		18.9		9.0	
hexyl hexanoate[+]	206.9		199.3		228.3		147.8	
alpha-farnesene[+]	272.2		280.0		335.9		205.0	

[1]Compounds are presented in the order of elusion from the gas-chromatograph. Means are duplicate analyses of two replications (week 0) with data for sorbate addition pooled.
[2]AIR = atmospheric air (2kGy), O2 = oxygen-flush (2kGy), N2 = nitrogen-flush (2kGy), RAW = atmospheric air (0kGy).
[+]Effect of sorbate significant ($P<0.05$).
*Kovats retention index (RI) values are present for unidentified or tentatively identified flavor compounds.

Table 8. Effect of sorbate on initial contents of selected volatile cider flavor compounds[1]

	GC peak areas (week 0) 0% sorbate		0.1% sorbate	
butanol[+]	57.3	a	97.5	b
propyl hexanoate[+]	138.2	a	398.8	b
nonanal[+]	90.0	a	194.0	b
heptyl acetate[+]	39.5	a	209.6	b
hexyl butanoate[+]	1017.9	a	1411.0	b
hexyl hexanoate[+]	125.5	a	265.7	b
alpha-farnesene[+]	185.1	a	361.5	b

[1]Means are duplicate analyses of two replications (Week 0) with data for gas environment pooled.
[+]Effect of sorbate significant ($P<0.05$).

Table 9. Interaction of gas environment and sorbate addition for initial contents of butanol[1]

Gas environment[2] (irradiation dose)	Initial GC peak area (week 0) 0% sorbate	0.1% sorbate
Air (2kGy)	84.4 ax	69.5 ax
N2 (2kGy)	44.7 ax	56.4 ax
O2 (2kGy)	72.3 ax	238.0 by
Raw (0kGy)	27.7 ax	26.0 ax

[1]Means are duplicate analyses of two replications. Means followed by different letters within the same row are significantly different among sorbate addition ($P<0.05$; a,b). Means followed by different letters within the same column are significantly different among gas environments ($P<0.05$; x,y).
[2]AIR = atmospheric air (2kGy), O2 = oxygen-flush (2kGy), N2 = nitrogen-flush (2kGy), RAW = atmospheric air (0kGy).

Effect of storage

Gas chromatograph (GC) peak areas were again plotted versus storage time in order to calculate slopes, i.e. the change in flavor intensity, for each principal flavor compound. As a result, a treatment variable was removed and the effects of only gaseous environment and sorbate addition remained. Since the greatest change in intensity of GC peak area takes place between week 0 and week 1 of storage, GC areas were again plotted on a logarithmic scale in order to calculate a more linear slope. The rate of change (slope) for each volatile flavor compound, SS, pH and TA was calculated and statistical analysis was completed on the slopes in order to determine the effects of gas environment and sorbate during storage.

Principle Component Analysis

PCA was again helpful to group the volatile flavor compounds, SS, pH and TA into five principle components. Linear slopes of analytical data (soluble solids, pH, and titratable acidity) and slopes of the log GC area vs. storage week were loaded onto the PCA function. The first five principle components (PC) accounted for more than 70% of the total variability

in the data set. PC-1 (31.2%) contained 21 flavor compounds, PC-2 (16.9%) contained SS, TA and 9 flavor compounds, PC-3 (12.2%) contained pH and 5 flavor compounds, PC-4 (5.3%) contained 2 flavor compounds and PC-5 (5.1%) contained 3 compounds (Figure 10).

As in Experiment I, a majority of the characteristic apple cider volatile flavor compounds, especially acetates and butanoates, loaded onto PC-1 while the major alcohols were grouped into PC-2. Each principle component did not contain an exclusive type of flavor compound, nor did the compounds exhibit the same response to gas environment and sorbate addition. The effects of gas environment and sorbate addition, therefore, will be discussed separately.

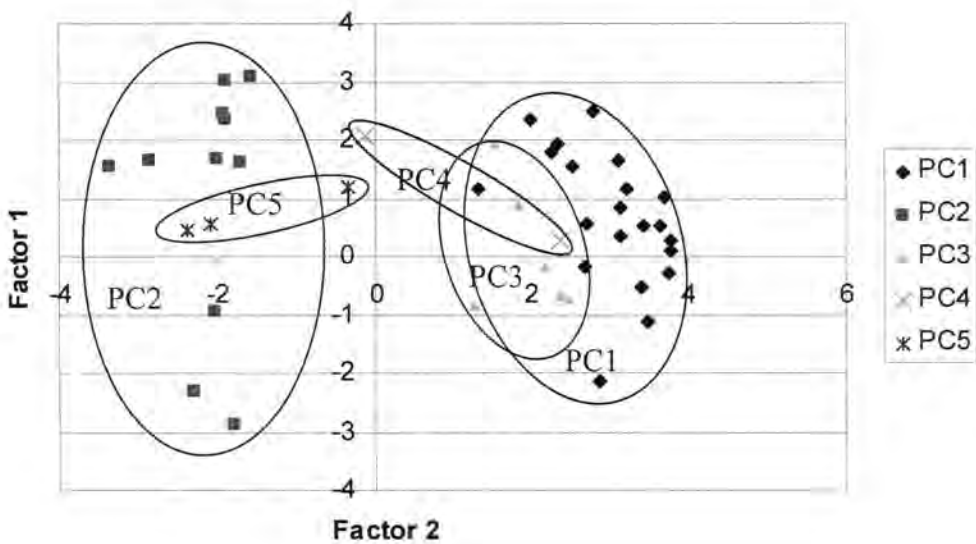


Figure 10. Plot of the PCA of apple cider showing associations between volatile flavor compounds and analytical data. Vector coordinates representing individual flavor compounds, soluble solids, pH or titratable acidity signify pooled responses for all processing treatments (irradiation, gas environment and sorbate addition) using the rates of change during seven weeks of refrigerated storage for two replications.

Effect of gas environment

A comparison of the slopes for each of the gas environments illustrates the retention of flavor compounds in irradiated cider compared to untreated (raw) cider during seven weeks of storage following irradiation treatment. Based on the data shown in Table 10, eight of the loadings in PC-1, five in PC-2 and one in PC-3 showed significant effects of the applied gas environments. In the case of SS, pH, ethyl-2-methyl propionate, ethyl hexanoate, and hexanol, raw cider had significantly more negative slopes (higher rate of loss) than all three irradiated treatments. For these compounds, irradiation helped maintain volatile flavor compounds during storage. The degradation caused by microbial and enzymatic reactions was reduced.

An opposite trend can be noted when slopes of O_2 and raw samples are compared directly. In the case of TA, methanol, propanol, 2-methyl butyl acetate, hexyl propionate, butyl butanoate and decanal, raw samples had significantly less negative slopes (lower rates of loss) than O_2 samples. It is possible that an oxygen-flush environment promotes oxidation reactions that are unfavorable to the preservation of characteristic apple cider flavor compounds. Moreover, samples exposed to atmospheric air behaved more similar to nitrogen-flush samples (than oxygen-flush samples) for characteristic apple cider flavor compounds such as ethyl-2-methyl butanoate and butyl butanoate. Since oxygen-flush cider had the most negative slopes (greatest loss in storage) for characteristic flavor compounds, atmospheric air and nitrogen-flush gas environments prior to irradiation treatment are preferred.

Table 10. Effects of gas environments on the retention of analytical data and volatile flavor compounds of apple cider during seven weeks of refrigerated storage[1]

	SLOPE (log GC peak area/week)							
Gas environment[2]	AIR		O2		N2		RAW	
Irradiation dose	2kGy		2kGy		2kGy		0kGy	
COMPONENT 1 (PC-1)								
1-methyl propyl acetate	-0.130		-0.135		-0.145		-0.182	
butyl acetate	-0.074		-0.237		-0.155		-0.066	
2-methyl butyl acetate*	-0.032	c	-0.250	ab	-0.134	bc	-0.027	c
3-pentyl acetate	-0.118		-0.153		-0.156		-0.164	
pentyl acetate	-0.084		-0.110		-0.137		-0.103	
hexyl acetate	-0.073		-0.143		-0.133		-0.107	
ethyl-2-methyl propionate*	0.310	b	0.322	b	0.307	b	-0.050	a
butyl propionate	-0.033		-0.109		-0.108		-0.035	
hexyl propionate*	-0.122	a	-0.142	a	-0.019	b	-0.036	b
ethyl butanoate	-0.059		-0.022		0.081		0.073	
ethyl-2-methyl butanoate*	-0.177	b	-0.289	a	-0.206	b	-0.283	a
propyl butanoate	-0.005		-0.088		-0.010		-0.005	
butyl butanoate*	-0.053	b	-0.092	a	-0.053	b	0.024	c
butyl-2-methyl butanoate	0.002		-0.025		-0.018		0.004	
pentyl butanoate	-0.108		-0.106		-0.109		-0.107	
hexyl butanoate	-0.118		-0.162		-0.134		-0.126	
hexyl-2-methyl butanoate	-0.059		-0.087		-0.091		-0.063	
2-hexenal	-0.208		-0.127		-0.196		-0.137	
decanal*	-0.019	b	-0.120	a	0.035	bc	0.090	c
methanol*	0.491	a	0.511	ab	0.578	b	0.669	c
hexanol	0.037	b	0.054	b	0.052	b	0.004	a
COMPONENT 2 (PC-2)								
SS*	0.005	b	0.035	b	0.025	b	-0.200	a
TA*	0.007	a	0.003	a	0.011	a	0.033	b
heptyl acetate*	0.032		0.006		0.055		0.070	
ethyl propionate	0.051		0.032		0.042		0.080	
ethyl hexanoate*	-0.045	bc	-0.054	b	-0.020	c	-0.150	a
benzaldehyde	0.033	b	-0.083	ab	-0.045	b	-0.176	a
t-2-nonenal	0.071		0.116		0.077		0.125	
p-allyl anisole	-0.031		-0.053		-0.029		0.006	
ethanol	0.263		0.205		0.326		0.401	
propanol	0.079	ab	0.051	a	0.158	bc	0.217	c
butanol	0.025		0.054		0.075		0.068	

Table 10 (continued)[1]

	SLOPE (log GC peak area/week)			
Gas environment[2]	AIR	O2	N2	RAW
Irradiation dose	2kGy	2kGy	2kGy	0kGy
COMPONENT 3 (PC-3)				
pH	0.002 b	0.001 b	-0.001 b	-0.012 a
benzyl acetate	0.030	0.030	0.065	0.027
methyl-2-methyl butanoate	0.026	0.029	0.048	0.079
hexyl hexanoate	-0.119	-0.075	-0.138	-0.098
alpha-farnesene	-0.205	-0.099	-0.155	-0.129
2-decenal	0.012	0.006	0.016	0.019
COMPONENT 4 (PC-4)				
propyl hexanoate*	-0.007	-0.043	0.023	-0.003
nonanal	0.023	-0.014	0.008	-0.006
COMPONENT 5 (PC-5)				
isopropyl-2-methyl butanoate	0.047	0.008	0.020	0.018
1-octen-3-ol	-0.004	-0.034	0.016	0.028
1-decanol	-0.060	-0.064	-0.049	-0.023

[1]Means are duplicate analyses of two replications with data for sorbate addition pooled. Means followed by different letters within the same row are significantly different from each other ($P<0.05$).
[2]AIR = atmospheric air (2kGy), O2 = oxygen-flush (2kGy), N2 = nitrogen-flush (2kGy), RAW = atmospheric air (0kGy).
[3]SS = soluble solids (% sucrose at 20°C). Slope = decrease in SS/week in storage.
[4]TA = titratable acidity (g malic acid/100mL cider). Slope = decrease in TA/week in storage.
*Effect of interaction between gas environment and sorbate significant ($P<0.05$).

Effect of sorbate addition

When comparing gas chromatograms from week 0 and week 7, an obvious difference can be identified between raw apple cider with and without preservative for week 7 compared to week 0 (Appendix C, Appendix D). Week 0 chromatograms were quite similar for raw cider with and without preservative. A slight difference can be noted in overall intensity of the major peaks and around a retention time of 18.6 minutes where sorbic acid provides an additional peak (Appendix C.1). Week 7 chromatograms are quite different from

week 0 in the absence of sorbate. A large peak around 1.5 minutes overwhelms the graph and the GC area (y-axis) exceeds 4000 (Appendix D.2). The overall flavor profile of raw apple cider with sorbate (Appendix D.1), however, remains similar to that of week 0.

The effect of potassium sorbate was very apparent as samples were compared directly between the presence and absence of sorbate (Table 11). Only twelve of the volatile flavor compounds did not exhibit a significant effect of sorbate of on the rates of change during storage. Slopes were more negative (higher rates of loss) for a majority of the characteristic volatile compounds which loaded onto PC-1. The opposite case, in which samples with sorbate had significantly greater slopes, was apparent for ethyl 2-methyl propionate, methanol, and hexanol. The presence of sorbate, therefore, significantly reduces the loss of characteristic apple cider volatile flavor compounds while the absence of sorbate increases the production of alcohols during seven weeks of refrigerated storage. These findings support those of Baroody and McLellan (1986) since sorbate was found to inhibit the growth of yeast and molds. As observed in Experiment I, fermentation or other degradation reactions diminish the apple or fruity compounds that give apple cider desirable quality and the production of alcohol serves an indicator of fermentation. Sorbate increases the shelf-life of cider (i.e. minimizes the effects of storage) by increasing the time which characteristic apple cider flavor profiles remain.

Analytical measurements are also helpful in supporting the effect of sorbate addition. Titratable acidity increased at a greater slope in the absence of sorbate than it did in the presence of 0.1% sorbate. Soluble solids increased in the presence of sorbate but decreased in the absence of sorbate. These opposing trends are expected as a result of a fermentative process as sugar is converted into acids and alcohols.

Interactions of Gas Environment and Sorbate Addition

The interaction of gas environment and sorbate for slope values was observed for SS, TA and thirteen volatile compounds (Table 12). Overall, in the presence of sorbate, differences between gas environments were not as significant as they were in the absence of sorbate. This supports, once again, that sorbate minimizes storage affects of raw cider and irradiated apple cider exposed to different gas environments. Volatile flavor analysis results provided methanol peaks only for apple cider that did not contain sorbate. These compounds may serve as indicators of fermentation or degradation reactions which increase in the absence of sorbate.

Coelution of Sorbic Acid and Nonanal

GC-MS confirmed that sorbic acid and nonanal coelute from the column on the same chromatogram peak. It is sometimes difficult, therefore, to quantify nonanal since the two compounds may combine to form one oversized peak. One compound with a large sensory impact may be masked by other compounds with low sensory impact but similar retention times (Foley et al., 2002).

Table 11. Effects of sorbate addition on the retention of analytical data and volatile flavor compounds of apple cider during seven weeks of refrigerated storage[1]

	SLOPE (log GC peak area/week)			
	0% sorbate		0.1% sorbate	
COMPONENT 1 (PC-1)				
1-methyl propyl acetate	-0.235	a	-0.060	b
butyl acetate	-0.245	a	-0.020	b
2-methyl butyl acetate*	-0.198	a	-0.023	b
3-pentyl acetate	-0.254	a	-0.042	b
pentyl acetate	-0.186	a	-0.031	b
hexyl acetate	-0.219	a	-0.009	b
ethyl-2-methyl propionate*	0.222	b	ND	a
butyl propionate	-0.143	a	0.001	b
hexyl propionate*	-0.205	a	-0.001	b
ethyl butanoate	-0.049		0.086	
ethyl-2-methyl butanoate*	-0.410	a	-0.068	b
propyl butanoate	-0.057		0.003	
butyl butanoate*	-0.093	a	0.006	b
butyl-2-methyl butanoate	-0.028	a	0.009	b
pentyl butanoate	-0.233	a	0.018	b
hexyl butanoate	-0.247	a	-0.024	b
hexyl-2-methyl butanoate	-0.131	a	-0.020	b
2-hexenal	-0.327	a	-0.007	b
decanal*	-0.088	a	0.037	b
methanol*	0.562	b	ND	a
hexanol	0.083	b	-0.009	a
COMPONENT 2 (PC-2)				
SS[2]*	-0.124	a	0.057	b
TA[3]*	0.023	b	0.004	a
heptyl acetate*	0.080	b	0.001	a
ethyl propionate	0.091		0.012	
ethyl hexanoate*	-0.138	a	0.004	b
benzaldehyde	-0.166	a	-0.020	b
t-2-nonenal	0.122		0.073	
p-allyl anisole	-0.022		-0.031	
ethanol	0.299	b	ND	a
propanol	0.186		0.066	
butanol	0.105	b	0.006	a

Table 11 (continued)[1]

	SLOPE (log GC peak area/week)			
	0% sorbate		0.1% sorbate	
COMPONENT 3 (PC-3)				
pH	0.003	b	-0.008	a
benzyl acetate	0.019		0.057	
methyl-2-methyl butanoate	0.068		0.024	
hexyl hexanoate	-0.160	a	-0.055	b
alpha-farnesene	-0.197	a	-0.097	b
2-decenal	0.005		0.022	
COMPONENT 4 (PC-4)				
propyl hexanoate*	-0.003		-0.012	
nonanal	-0.017		0.023	
COMPONENT 5 (PC-5)				
isopropyl-2-methyl butanoate	0.041	b	0.006	a
1-octen-3-ol	0.054	b	-0.051	a
1-decanol	-0.043		-0.055	

[1]Means are duplicate analyses of two replications with data for gas environment pooled. Means followed by different letters within the same row are significantly different from each other ($P<0.05$).

[2]SS = soluble solids (% sucrose at 20°C).

[3]TA = titratable acidity (g malic acid/100mL cider).

*Effect of interaction between gas environment and sorbate significant ($P<0.05$).

Table 12. Interaction of gas environment and sorbate addition for analytical data and cider flavor compounds [1]

	SLOPE (log GC peak area/week)			
Gas environment[2]	AIR	N2	O2	RAW
soluble solids (SS)				
0% sorbate	-0.016 ay	-0.024 ay	0.001 ay	-0.459 ax
0.1% sorbate	0.026 ax	0.074 ax	0.068 ax	0.058 bx
titratable acidity (TA)				
0% sorbate	0.012 bx	0.023 by	0.004 ax	0.054 bz
0.1% sorbate	0.001 ax	-0.001 ax	0.003 axy	0.012 ay
2-methyl butyl acetate				
0% sorbate	-0.050 ay	-0.234 ay	-0.476 ax	-0.033 ay
0.1% sorbate	-0.013 ax	-0.033 ax	-0.024 bx	-0.021 ax
heptyl acetate				
0% sorbate	0.008 ax	0.118 byz	0.026 axy	0.168 bz
0.1% sorbate	0.055 ax	-0.009 ax	-0.014 ax	-0.027 ax
ethyl-2-methyl propionate				
0% sorbate	0.310 by	0.307 by	0.322 by	-0.050 ax
0.1% sorbate	ND ax	ND ax	ND ax	ND ax
hexyl propionate				
0% sorbate	-0.270 ax	ND az	-0.278 ax	-0.065 ay
0.1% sorbate	0.027 bx	-0.019 ax	-0.005 bx	-0.006 bx
ethyl-2-methyl butanoate				
0% sorbate	-0.348 ay	-0.401 ay	-0.563 ax	-0.328 ay
0.1% sorbate	-0.007 by	-0.012 by	-0.014 by	-0.238 bx
butyl butanoate				
0% sorbate	-0.117 ay	-0.108 ay	-0.179 ax	0.032 az
0.1% sorbate	0.012 bx	0.002 bx	-0.004 bx	0.016 ax
ethyl hexanoate				
0% sorbate	-0.072 ayz	-0.050 az	-0.098 ay	-0.332 ax
0.1% sorbate	-0.017 bx	0.010 bxy	-0.009 bxy	0.032 by
propyl hexanoate				
0% sorbate	-0.009 axy	0.064 bz	-0.079 ax	0.014 ayz
0.1% sorbate	-0.004 ax	-0.017 ax	-0.006 bx	-0.020 ax
hexanal				
0% sorbate	-0.241 ax	-0.155 ax	-0.210 ax	-0.164 bx
0.1% sorbate	0.013 by	-0.026 ay	-0.022 by	-0.384 ax
decanal				
0% sorbate	-0.072 ay	0.027 az	-0.221 ax	ND
0.1% sorbate	0.034 bxy	0.044 axy	-0.019 bx	0.090 y

Table 12 (continued) [1]

Gas environment[2]	SLOPE (log GC peak area/week) AIR	N2	O2	RAW
methanol				
0% sorbate	0.491 bx	0.511 bx	0.578 by	0.669 bz
0.1% sorbate	ND ax	ND ax	ND ax	ND ax
1-octanol				
0% sorbate	ND ax	0.416 by	ND ax	ND ax
0.1% sorbate	ND ax	ND ax	ND ax	ND ax
RI 1136*				
0% sorbate	-0.255 ax	-0.226 ax	-0.230 ax	-0.220 ax
0.1% sorbate	-0.002 by	-0.008 by	-0.257 ax	-0.028 by

[1]Means are duplicate analyses of two replications. Means followed by different letters within the same column are significantly different among sorbate addition ($P<0.05$; a,b). Means followed by different letters within the same row are significantly different among gas environments ($P<0.05$; x,y,z).
[2]AIR = atmospheric air (2kGy), O2 = oxygen-flush (2kGy), N2 = nitrogen-flush (2kGy), RAW = atmospheric air (0kGy).
ND = not detected.
*Kovats retention index (RI) values are present for unidentified flavor compounds.

Conclusions of Experiment II

Based on the data from Experiment II, the effects of gas environment and sorbate addition are important for retaining characteristic apple cider flavor and quality attributes. Based on the trends in analytical data (SS and TA), sorbate addition minimizes changes in soluble solids and acidity contents compared to cider without sorbate as a result of inhibited yeast growth. When comparing the three gaseous environments, acidity measurements suggest that an oxygen-flush environment minimizes the increase in acidity in cider with sorbate, even following seven weeks of storage.

Microbiological data based on yeast and aerobic plate counts is also valuable in following apple cider quality changes which occur during storage as well as the effects of gas environments and sorbate addition. Initial microbial counts indicated the importance of

irradiation to decrease microorganism populations prior to storage. Yeast populations increased rapidly in the absence of sorbate, which indicates the importance of this compound to limit fermentation and extend the shelf life of apple cider. By week 7, aerobic bacteria grew to the level of 10^6 in oxygen-flush cider, which was higher than counts for raw cider.

Volatile flavor analysis is a useful tool for monitoring changes in characteristic apple cider compounds which occur during storage. In the absence of sorbate, characteristic apple cider esters have a greater rate of loss than in cider with sorbate. For major characteristic apple cider flavor compounds such as 2-methyl butyl acetate, ethyl-2-methyl butanoate and butyl butanoate, oxygen-flush treatments had the greatest loss in GC peak area (for irradiated samples) during storage. For this reason, atmospheric air or nitrogen-flush conditions exposed to cider before irradiation treatment are favored over oxygen-flush conditions.

GENERAL CONCLUSIONS

Analytical measurements and volatile flavor profiles were successful in monitoring the quality attributes of apple cider throughout refrigerated storage. The combination of irradiation, sorbate addition, packaging materials and/or gas environments are possible treatments which can be implemented during apple cider production to limit pathogenic organisms, extend the shelf life of apple cider, and decrease undesirable flavor loss.

Based on the results of this experiment, many recommendations are suggested to minimize the loss of characteristic flavor quality and extend the shelf-life of refrigerated apple cider. Cider producers should implement HACCP principles in order to prevent cross contamination from equipment or large microbial loads in fresh apple cider. The addition of 0.1% potassium sorbate to fresh apple cider will increase the product's shelf-life. In order to achieve the necessary 5-$\log_{10}$ reduction in pathogens, non-thermal electron beam irradiation can be used as an alternative to heat pasteurization. Polystyrene and nitrogen-flush or atmospheric air packaging conditions will also minimize the loss of characteristic apple cider flavor profiles during extended storage.

The application of electron beam irradiation as an alternative to heat pasteurization involves many stipulations. First of all, the process must remain economical and efficient for apple cider producers. The proper regulatory measures must also be considered in order to provide safe and acceptable apple cider. Most importantly, consumer concerns and acceptance must be taken into accounts in all areas of implementation.

Future research in this area should involve sensory evaluation of cider under similar conditions as the experiments in this study. Correlations should be made between analytical measurements, volatile flavor profiles and sensory data. A comparison between pasteurized

and irradiated apple cider will also be helpful to evaluate the positive and negative impacts of both processing treatments, under optimal processing and storage conditions. Other possible procedures include gas chromatography-olfactometry (GCO) to define what desirable and undesirable aromas are caused by each characteristic flavor compound as related to sensory findings. Extended research may be attempted to identify and study the natural yeasts present in apple cider which are resistant to the process of irradiation. Finally, the antioxidant potential or ability of apple cider to accept free radical formation as a result of gas environment and/or irradiation treatment may also be important to the effects of such processing and storage conditions.

APPENDIX A. CIDER COMPOSITION AND QUALITY

1. General composition of apple cider

Chemical Composition [a]	Natural Sugars
water (86% to 88%)	fructose (4.5% to 8.5%)
carbohydrates (11% to 12%)	sucrose, (1.5% to 4.5%)
fat (0.25%)	glucose (1.2% to 2%)
protein (0.25%)	**Acid Composition**
fiber (0.5%)	malic acid (0.15% to 1.1%)
ascorbic acid or vitamin C (3 mg to 30 mg/100 gm)	citric acid (trace amounts)

[a] Expressed as a range or an average

2. Primary flavor characteristics of Pennsylvania apple cultivars based on sugar/acid (S/A) ratios.

Group I	Group II	Group III	Group IV	Group V
Relatively acid or tart (S/A<20) [a]	**Balanced flavor, sweet and tart (S/A 20 to 40)**	**Relatively sweet (S/A>40)**	**Very aromatic**	**Very astringent; high in tannins**
Granny Smith	Baldwin	Red Delicious	Cortland	Crabapples
Idared	Cortland	Grimes Golden	Golden Delicious	Immature apples
Jonathan	Golden Delicious	Fuji	Empire	
Northern Spy	Empire	Gala	Gravenstein	
R.I. Greening	Gravenstein		Jonagold	
Stayman	Mutsu		Macoun	
Wealthy	McIntosh		McIntosh	
Winesap	Rome Beauty		Wealthy	
	Spartan		Winter Banana	
	York Imperial			
	Braeburn			

[a] Ratio of sugar to acid (S/A) is less than 20 (<20)

3. Relative initial quality and shelf life of cider

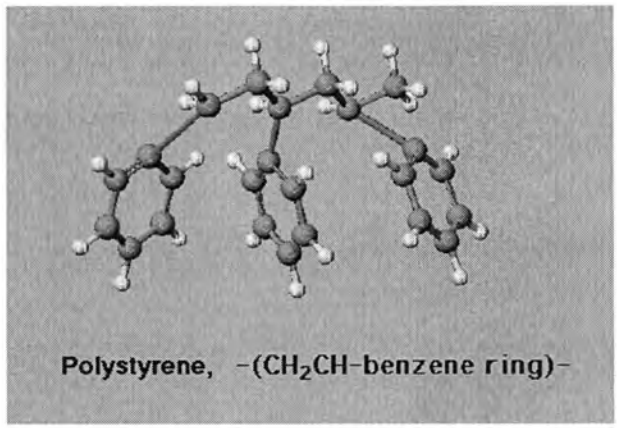
Initial flavor quality when not preserved and when preserved by various methods					
Unpasteurized/ no preservatives	0.1% potassium sorbate added	0.1% sodium benzoate added	Heated until pasteurized	Heated until sterile/canned	Frozen/stored at 0° F
Very high	High	Good [a]	Good [b]	Fair [c]	Very high
Relative shelf life as influenced by preservation method and storage temperature					
Stored 2 - 3 days at 46° - 50° F	Stored 1 - 2 wk at 46° - 50° F	Stored 1 - 2 wk at 46° - 50° F	Stored 1 -2 mo at 46° - 50° F	Stored 1 - 3 yr at room temp	Stored 2 - 3 yr at 0° F
Stored 12 - 14 days at 32° - 36° F	Stored 2 - 3 mo at 32° - 36° F	Stored 2 - 3 mo at 32° - 36° F	Stored 3 -6 mo at 32° - 36° F		

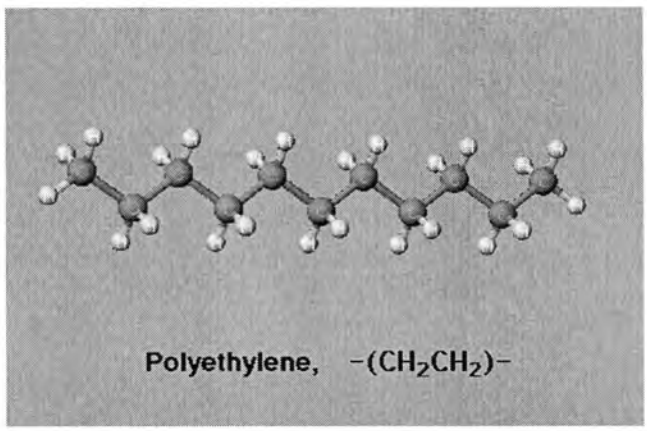
[a] Cider may have a slightly bitter flavor.
[b] Cider may have a slightly cooked flavor.
[c] Cider will have a detectable cooked flavor.

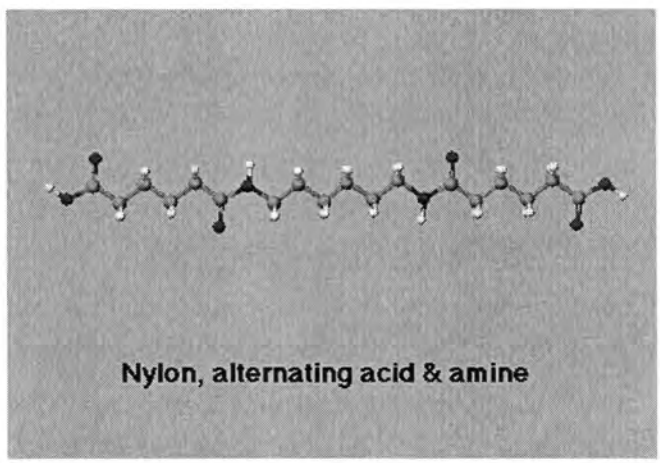
Source: PennState; *Pennsylvania Tree Fruit Production Guide*, 2003.

APPENDIX B. PACKAGING MATERIALS

Chemical structure of the three polymeric films used in Experiment I



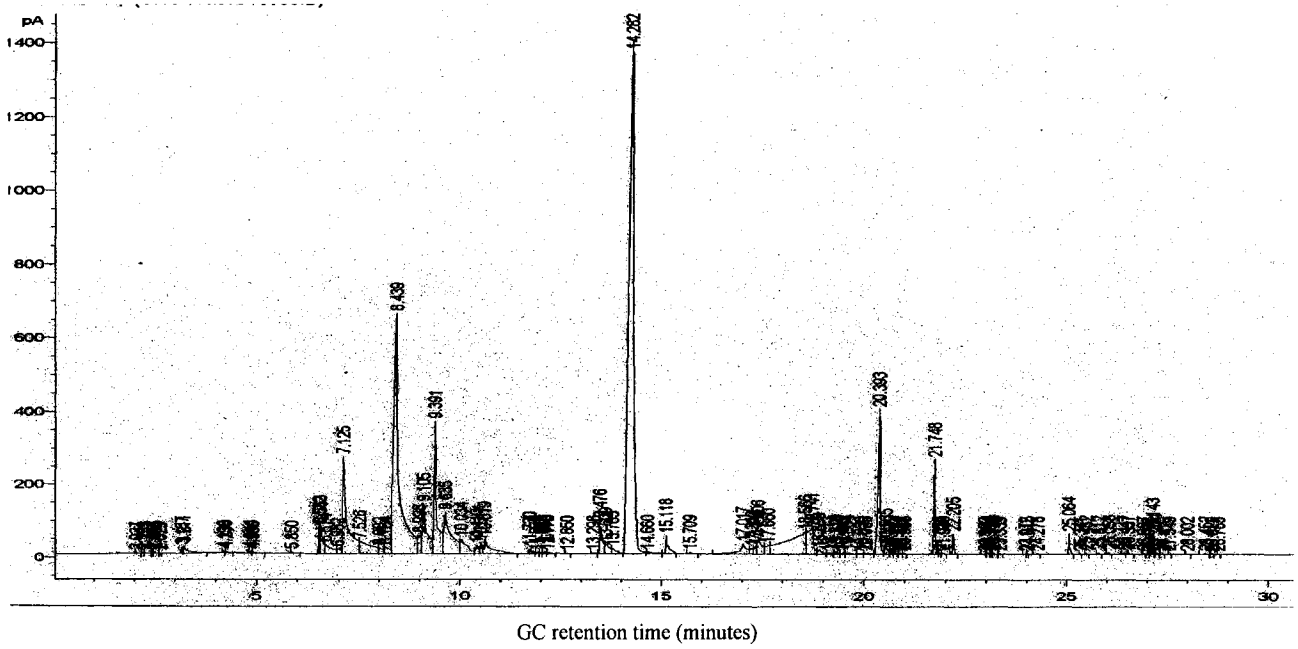




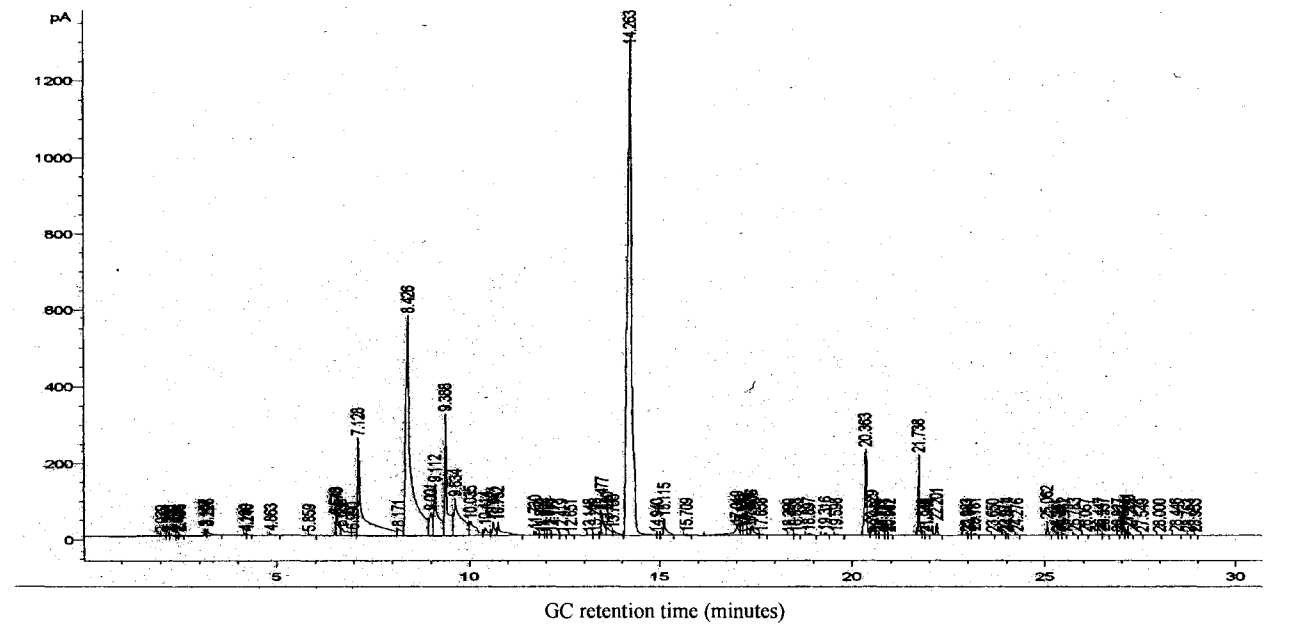
Source: http://www2.polito.it/centri/cemed/sistemaperiodico/s13/e13_1_02.html

APPENDIX C. WEEK 0 GAS CHROMATOGRAMS

(1)



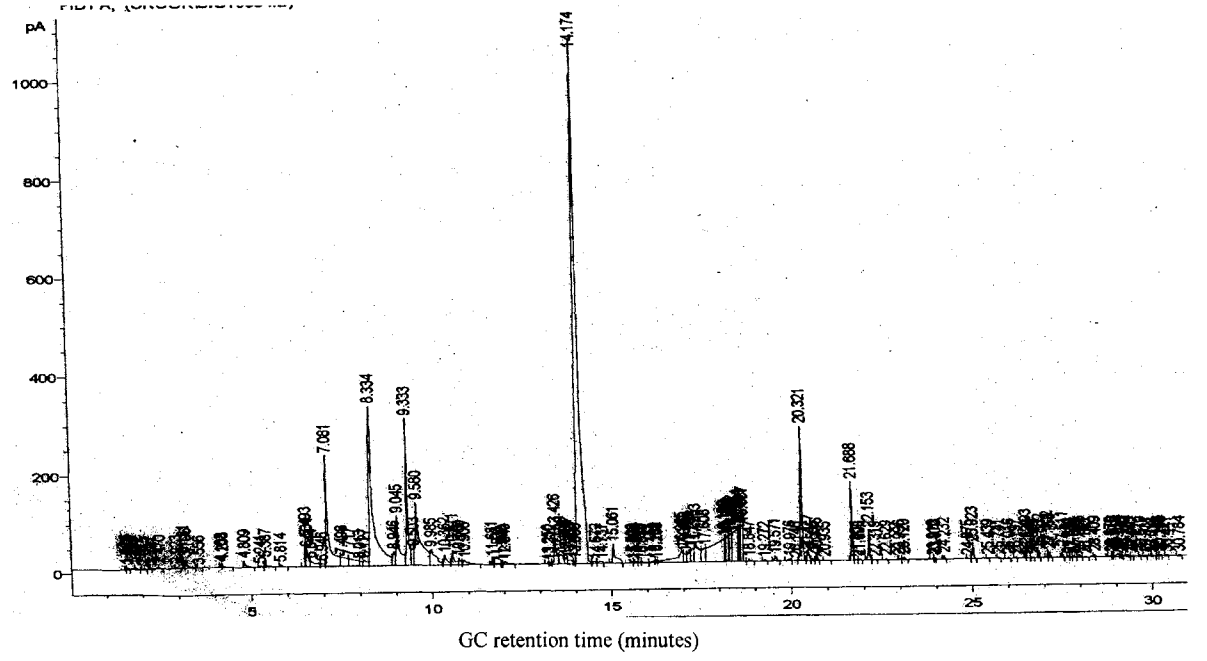
(2)



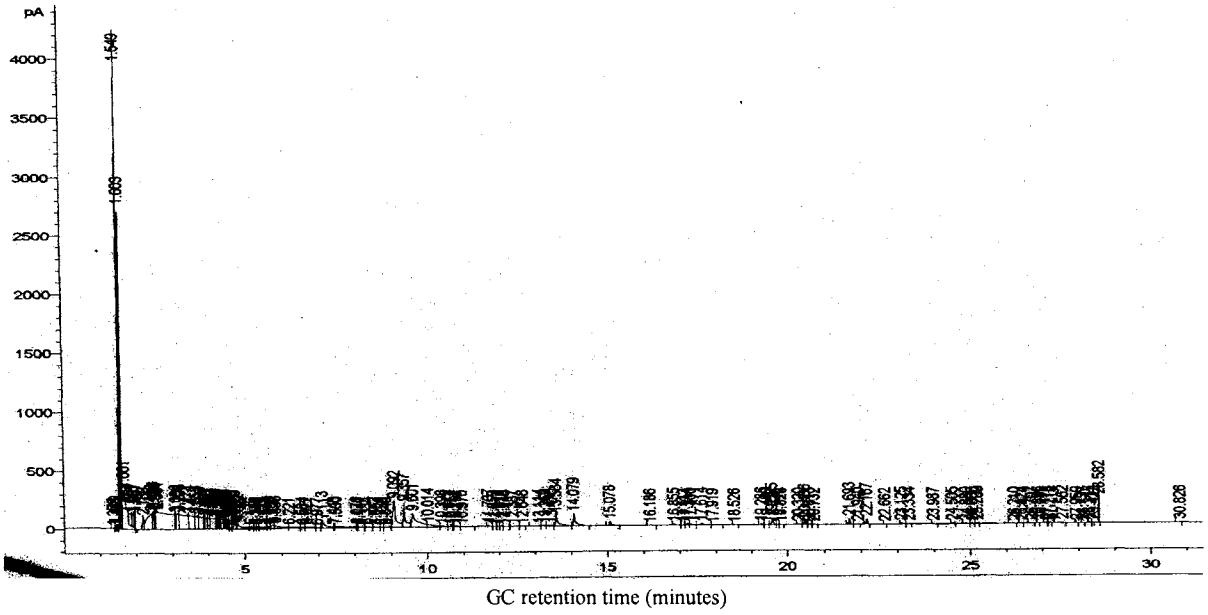
Gas Chromatograms from Experiment II (Week 0) for raw apple cider (1) with 0.1% sorbate and (2) without sorbate.

APPENDIX D. WEEK 7 GAS CHROMATOGRAMS

(1)



(2)



Gas Chromatograms from Experiment II (Week 7) for raw apple cider (1) with 0.1% sorbate and (2) without sorbate.

REFERENCES CITED

Arya, S.S. Stability of sorbic acid in aqueous solutions. *J. Agric. Food Chem.*, **1980**, 28, 1246-1249.

Askar, A. Flavor changes during processing and storage of fruit juices; I. Markers for processed and stored fruit juices. *Fruit Proc.*, **1999a**, 9, 236-244.

Askar, A. Flavor changes during processing and storage of fruit juices; II. Interactions with packaging materials. *Fruit Proc.*, **1999b**, 9, 432-439.

Ayhan, Z.; Yeom, H.W.; Zhang, Q.H.; Min, D.B. Flavor, color, and vitamin C retention of pulsed electric field processed orange juice in different packaging materials. *J. Agric. Food Chem.*, **2001**, 49, 669-674.

Azuma, K.; Hirata; T.; Tsunoda, H.; Ishitani, T.; Tanaka, Y. Identification of the volatiles from low density polyethylene film irradiated with an electron beam. *Agric. Biol. Chem.*, **1983**, 47, 855-860.

Azuma, K.; Tsunoda, H.; Hirata, T.; Ishitani, T.; Tanaka, Y. Effects of the conditions for electron beam irradiation on the amounts of volatiles from irradiated polyethylene film. *Agric. Biol. Chem.*, **1984**, 49, 2009-2015.

Baroody, T.; McLellan, M.R. The effect of pH, acidity, and low level sorbate on the shelflife of inoculated apple juice. *J. Food Qual.*, **1986**, 9, 415-423.

Bettini, M.F.M.; Shaw, P.E.; Lancas, F.M. Sensory and analytical evaluations of Brazilian orange juices and aromas. *Fruit Proc.*, **1998**, 8, 283-287.

Bills, S.; Restaino, L.; Lenovich, L.M. Growth response of an osmotolerant sorbate-resistant yeast, Saccharomyces rouxii, at different sucrose and sorbate levels. *J. Food Prot.*, **1982**, 45, 1120-1124.

Boylston, T.D.; Kupferman, E.M.; Foss, J.D.; Buering, C. Sensory quality of *Gala* apples as influenced by controlled and regular atmosphere storage. *J. Food Qual.*, **1994**, 17, 477-494.

Boylston, T.D.; Reitmeier, A.A.; Moy, J.H.; Mosher, G.A.; Taladriz, L. Sensory quality and nutrient composition of three hawaiian fruits treated by X-irradiation. *J. Food Qual.*, **2002**, 25, 419-433.

Boylston, T.D.; Wang, H.; Reitmeier, C.A.; Glatz, B. Effects of processing treatment and sorbate addition on the flavor characteristics of apple cider. *J. Agric. Food Chem.*, **2003**, 51, 1924-1931.

Briston, J.H.; Katan, L.L. *Plastics films*, 3rd Ed.; John Wiley and Sons Inc.: New York, **1988**; 434 pp.

Buchalla, R.; Schuttler, C.; Bogl, K.W. Effects of ionizing radiation on plastic food packaging materials: a review; Part 1. Chemical and physical changes. *J. Food Prot.*, **1993**, 56, 991-997.

Carr, J.G. Spoilage of cider. *J. Appl. Bacteriol.*, **1984**, 57, 1320-1450.

Comes, J.E.; Beelman, R.B. Addition of fumaric acid and sodium benzoate as an alternative method to achieve 5-log reduction of Escherichia coli 0157:H7. *J. Food Prot.*, **2002**, 65, 476-483.

Cummins, A.; Reitmeier, C.; Wilson, L.; Glatz, B. A survey of apple cider production practices and microbial loads in cider in the state of Iowa. *Dairy, Food Environ. Sanitation*, **2002**, 22, 745-751.

Cunningham, D.G.; Acree, T.E.; Barnard, J.; Butts, R.M.; Braell, P.A. Charm analysis of apple volatiles. *Food Chem.*, **1986**, 19, 137-147.

Dauthy, M.E. *Fruit and vegetable processing*. FAO Agricultural Services Bulletin No. 119, **1995**.

Deak, T.; Beuchat, L.R. *Handbook of food spoilage yeasts*. CRC Press, Inc.: Boca Raton, FL, **1996**; 210 pp.

Deol, P. *Presence and growth of microorganisms in Iowa apple cider* [MSc thesis]. Ames, IA: Iowa State Univ., **2003**; 202 pp. Available from: Iowa State University Parks Library, Ames, IA.

Dimick, P.S.; Hoskin, J.C. Review of apple flavor - state of the art. *CRC Rev. Food Sci. Nutr.*, **1983**, 18, 387-409.

Durr, P.; Rothlin, M. Development of a synthetic apple juice odour. *Lebensm. Wiss. Technol*, **1981**, 14, 313-314.

Emard, L.O. Study of the apples and yeasts of various regions of Quebec from the viewpoint of commercial-scale production of good quality cider. *Agro Alimentaire*, **1974**, 1, 8-13.

FDA. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice; final rule. *Code of Fed. Reg.*: Jan. 19, **2001**. 66: 6137-6202.

Food and Drug Administration. *Code of Fed. Reg.* Title 21, 121.1.

FDA website: www.fda.gov; June 18, **2003.**

FDA. *Code of Fed. Reg.* Food Labeling: Warning and Notice Statement; Labeling of Juice Products (21 CFR Part 101 [Docket No. 97N-0524] RIN 0910-AA43) July 8, **1998**.

Fischer, T.L.; Golden, D.A. Fate of *Escherichia coli* 0157:H7 in ground apples used in cider production. *J. Food Prot.*, **1998**, 61, 1372-1374.

Flath, R. A.; Black, D.R.; Forrey, R.R.; McDonald, G.M.; Mon, T.R.; Teranishi, R. Volatiles in Gravenstein apple essence identified by gas chromatography-mass spectrometry. *J. Chromatog. Sci.*, **1969**, 7, 508-512.

Flath, R.A.; Black, D.R.; Guadadni, D.G.; McFadden, W.H.; Schultz, T.H. Identification and organoleptic evaluation of compounds in *Delicious* apple essence. *J. Agric. Food Chem.*, **1967**, 15, 29-35.

Foley, D.M.; Pickett, K.; Varon, J.; Lee, J.; Min, D.B.; Caporaso, F.; Prakash, A. Pasteurization of fresh orange juice using gamma irradiation: microbiological, flavor and sensory anaylses. *J. Food Sci.*, **2002**, 67, 1495-1501.

Gardner, P.T.; White, T.A.C.; McPail, D.B.; Duthie, G.G. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem.*, **2000**, 68, 471-474.

Gerschenson, L.N.; Alzamora, S.M.; Chirife, J. Stability of sorbic acid in model food systems of reduced water activity: sugar solutions. *J. Food Sci.*, **1986**, 51, 1028-1031.

Glidewell, S.M.; Deighton, N.; Goodman, B.A.; Hillman, J.R. Detection of irradiated food: a review. *J. Sci. Food Agric.*, **1993**, 61, 281-300.

Guichard, E.; Issanchou, S. Extraction of volatile compounds from raspberry by different methods: Application of principle component analysis to gas chromatograph data. *Sciences des Aliments*, **1983**, 3, 427-438.

Harrington, W.O.; Hills, C.H. Reduction of the microbial population of apple cider by ultraviolet irradiation. *Food Technol.*, **1968**, 22 (11), 1451-1454.

Hayes, D.J.; Fox, J.A.; Shogren, J.F. Experts and activists: how information affects the demand for food irradiation. *Food Policy*, **2002**, 27, 185-193.

Hopfenberg, H.B.; Ed. *Permeability of plastic films and coatings to gases, vapors, and liquids*. Plenum Press: New York; **1974;** 482 pp.

Ingham, S.C.; Schoeller, N.P. Acceptability of a multi-step intervention system to improve apple cider safety. *Food Res. Int.*, **2002**, 35, 611-618.

Jay, J.M. *Modern food microbiology*, 6th Ed.; Aspen Publ.: Gaithersburg, MD, **2000**; 679 pp.

Josephson, E.S. An historical view of food irradiation. *J. Food Safety*, **1983**, 5, 161-190.

Keller, S.E.; Merker, R.I.; Taylor, K.T.; Tan, H.L.; Melvin, C.D.; Chirtel, S.J.; Miller, A.J. Efficacy of sanitation and cleaning methods in a small apple cider mill. *J. Food Prot.*, **2002**, 65, 911-917.

Kozempel, M.; McAloon, A.; Yee, W. The cost of pasteurizing apple cider. *Food Technol.*, **1998**, 52 (1), 50-52.

Lea, A.G.H.; Timberlake, C.F. The phenolics of ciders: effect of processing conditions. *J. Sci. Food Agric.*, **1978**, 29, 484-492.

Lopez, M.L.; Lavilla, M.T.; Riba, M.; Vendrell, M. Comparison of volatile compounds in two seasons in apples: *Golden Delicious* and *Granny Smith*. *J. Food Qual.*, **1998**, 21, 155-166.

Lopez-Gonzalez, V.; Murano, P.S.; Brennan, R.E.; Murano, E.A. Sensory evaluation of ground beef patties irradiated by gamma rays versus electron beam under various packaging conditions. *J. Food Qual.*, **2000**, 23, 195-204.

Luedtke, A.N.; Powell, D.A. A review of North American apple cider-associated *E. coli 0157:H7* outbreaks, media coverage and a comparative analysis of Ontario apple cider producers' information sources and production practices. *Dairy, Food Environ. Sanitation*, **2002**, 22, 590-598.

Mangas, J.J.; Gonzalez, M.P.; Rodriguez, R.; Blanco, D. Solid-phase extraction and determination of trace aroma and flavour components in cider by GC-MS. *Chromatographia*, **1996**, 42, 101-105.

Mangas, J.J.; Dapena, E.; Rodriguez, M.S.; Moreno, J. Changes in pectic fractions during ripening of cider apples. *HortScience*, **1992**, 27, 328-330.

Mangas, J.J.; Moreno, J.; Picinelli, A.; Blanco-Gomis, D. Characterization of cider apple fruits according to their degree of ripening: a chemometric approach. *J. Agric. Food Chem.*, **1998**, 46, 4174-4178.

Mangas, J.J.; Rodriguez, R.; Suarez, B.; Picinelli, A.; Dapena, E. Study of the phenolic profile of cider apple cultivars at maturity by multivariate techniques. *J. Agric. Food Chem.*, **1999**, 47, 4046-4052.

Marquenie, D.; Michiels, C.W.; Impe, J.F.; Schrevens, E.; Nicolai, B.N. Pulsed white light in combination with UV-C and heat to reduce storage rot of strawberry. *Postharvest Biol. Technol.*, **2003**, 27, 21-35.

Marth, E.H.; Capp, C.M.; Hasenzahl, L.; Jackson, H.W.; Hussong, R.V. Degradation of potassium sorbate by *Penicillium* species. *J. Dairy Sci.*, **1966**, 49, 1197-1205.

Matich, A.J.; Rowan, D.D.; Banks, N.H. Solid-phase microextraction for quantitative headspace sampling of apple volatiles. *J. Anal. Chem.*, **1996**, 68, 4114-4118.

Matsui,T.; Mizumoto, S.; Kotani, A.; Imakura, H.; Shimoda, M.; Osajima, Y. Depression of sorption of volatile compounds into EVA film by electron beam irradiation. *J. Sci. Food Agric.*, **1990**, 50, 507-515.

Matsui, T.; Ono, A.; Shimoda, M.; Osajima, Y. Thermodynamic elucidation of depression mechanism on sorption of flavor compounds into electron beam irradiated LDPE and EVA films. *J. Agric. Food Chem.*, **1992**, 40, 479-483.

Mattheis, J.P.; Buchanan, D.A.; Fellman, J.K. Changes in apple fruit volatiles after storage in atmosphere inducing anaerobic metabolism. *J. Agric. Food Chem.*, **1991**, 39, 1602-1605.

Miller, N.J.; Rice-Evans, C.A. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chem.*, **1997**, 60, 331-337.

Murdock, D.; Hatcher, W. Growth of microorganisms in chilled orange juice. *J. Milk Food Technol.*, **1975**, 38, 393-396.

Olson, D.G. An overview of different types of irradiation (e-beam, gamma ray, and x-ray irradiation. http://www.extension.iastate.edu/foodsafety/rad/irradhome.html; June 12, **2003.**

Passy, N. Off-flavors from packaging materials in food products some case studies. In *Instrumental Analysis of Foods*; Charalambous, G.; Inglett, G., Eds.; Academic Press, Inc.: New York, **1983;** Vol. 1, 437 pp.

PennState College of Agricultural Sciences website. *Pennsylvania Tree Fruit Production Guide*. http://tfpg.cas.psu.edu/default.htm; July 28, **2003**.

Piyasena, P.; Rayner, M.; Bartlett, F.M.; Lu, E.; McKellar, R.C. Characterization of apples and apple cider produced by a Guelph area orchard. *Lebensm. Wiss. Technol.*, **2002**, 35, 367-372.

Plestenjak, A.; Hribar, J.; Vidrih, R.; Simcic, M. Optimum harvest date and post storage quality of apples. *Acta Horticulturae*, **1994**, 368, 641-645.

Plotto, A.; McDaniel, M.R.; Mattheis, J.P. Characterization of 'Gala' apple aroma and flavor: differences between controlled atmosphere and air storage. *Am. Soc. Hort. Sci.*, **1999**, 124, 416-423.

Poll, L. Evaluation of 18 apple varieties for their suitability for juice production. *J. Sci. Food Agric.*, **1981**, 32, 1081-1090.

Poll, L. Influence of storage temperature on sensory evaluation and composition of volatiles of *McIntosh* apple juice. *Lebensm. Wiss. Technol.*, **1983**, 16, 220-223.

Poll, L. The influence of apple ripeness and juice storage temperature on the sensory evaluation and composition (volatile and non-volatile components) of apple juice. *Lebensm. Wiss. Technol.*, **1985**, 18, 205-211.

Poll, L.; Flink, J.M. Influence of storage time and temperature on the sensory quality of single-variety apple juice. *Lebensm. Wiss. Technol.*, **1983**, 16, 215-219.

Product systems website (Modelli di strutture chimiche di polimeri): http://www2.polito.it/centri/cemed/sistemaperiodico/s13/e13_1_02.html; July 24, 2003.

Restaino, L; Komatsu, K.K.; Syracuse, M.J. Effects of acids on potassium sorbate inhibition of food-related microorganisms in culture media. *J. Food Sci.*, **1981**, 47, 134-138.

Roberts, D.D.; Pollien, P.; Milo, C. Solid-phase microextraction method development for headspace analysis of volatile flavor compounds. *J. Agric. Food Chem.*, **2000**, 48, 2430-2437.

Salame, M. The use of low permeation thermoplastics in food and beverage packaging. *Polymer Sci. Technol.*, **1974**, 6, 275-284.

Sanoner, P.; Guyot, S.; Marnet, N.; Molle, D.; Drilleau, J.F. Polyphenol profiles of French cider apple varieties. *J. Agric. Food Chem.*, **1999**, 47, 4847-4853.

Senkel Jr., I.A.; Henderson, R.A.; Jolbitado, B.; Meng, J. Use of hazard analysis critical control point and alternative treatments in the production of apple cider. *J. Food Prot.*, **1999**, 62, 778-785.

Sizer, C.E.; Waugh, P.L.; Edstam, S.; Ackermann, P. Maintaining flavor and nutrient quality of aseptic orange juice. *Food Technol.*, **1988** , 42 (6), 152-159.

Song, J.; Bangerth, F. Production and development of volatile aroma compounds of apple fruits at different times of maturity. *Acta Horticulturae*, **1994**, 368, 150-159.

Stinson, E.E.; Osman, S.F.; Huhtanen, C.N.; Bills, D.D. Disappearance of patulin during alcoholic fermentation of apple juice. *Appl. Environ. Microbiol.*, **1979**, 36, 620-622.

Supelco. Solid phase microextraction: theory and optimization of conditions. *Sigma-Aldrich Co.* Bulletin 923, **1998**.

Thaker, B.R.; Arya, S.S. Effect of sorbic acid on irradiation-induced sensory and chemical changes in sweetened orange juice and mango pulp. *Int. J. Food Sci. Technol.*, **1993**, 28, 371-376.

Thayer, D.W.; Rajkowski, K.T. Developments in irradiation of fresh fruits and vegetables. *Food Technol.*, **1999**, 53 (11), 62-65.

Travers, I.; Jacquet, A.; Brisset, A.; Maite, C. Relationship between the enzymatic determination of starch and the starch iodine index in 2 varieties of cider apple. *J. Sci. Food Agric.*, **2002**, 82, 983-989.

USDA National Agric. Stat. Service www.fas.usda.gov/htp/horticulture/Apples/apple01.pdf; May 19, **2003**.

Vidrih, R.; Hribar, J. Synthesis of higher alcohols during cider processing. *Food Chem.*, **1999**, 67, 287-294.

Wang, H.; Reitmeier, C.A.; Glatz, B.A.; Carriquiry, A.L. Mixed model analysis of sensory characteristics of irradiated apple cider. *J. Food Sci.*, **2003**, 68, 1498-1503.

Wang, H.; Reitmeier, C.A.; Glatz, B.A. Elimination of *Escherichia coli 0157:H7* in apple cider by electron beam irradiation. Minneapolis, MN: International Association for Food Protection 88th Annual Meeting, Abstract No. P018. p 49-50, **2001**.

Watada, A.E.; Abbott, J.A.; Hardenburg, R.E.; Lusby, W. Relationships of apple sensory attributes to headspace volatiles, soluble solids, and titratable acids. *Am. Soc. Hort. Sci.*, **1981**, 106, 130-132.

Williams, A.A.; Lewis, M.J.; Tucknott, O.G. The neutral volatile components of cider apple juices. *Food Chem.*, **1981**, 6, 139-151.

Willige, R.V.; Schoolmeester, D.; Ooij, A.V.; Linssen, J.; Voragen, A. Influence of storage time and temperature on absorption of flavor compounds from solutions by plastic packaging materials. *J. Food Sci.*, **2001**, 67, 2023-2031.

Wills, R.B.H.; McGlasson, W.B. Effect of storage temperature on apple volatiles associated with low temperature breakdown. *Hort. Sci.*, **1971**, 46, 115-120.

Wilson, C.L.; Ghaouth, A.E.; Upchurch, B.; Stevens, C.; Khan, V.; Droby, S.; Chalutz, E. Using a on-line UV-C apparatus to treat harvested fruit for controlling postharvest decay. *Hort Technol.*, **1997**, 7, 278-282.

Wolke, R.L. Food Irradiation. *Gastronomica*, **2002**, 2, 89-91.

Wright, J.R.; Sumner, S.S.; Hackney, C.R.; Pierson, M.D.; Zoecklein, B.W. A survey of Virginia apple cider producers' practices. *Dairy, Food, Environ. Sanitation*, **2000**, 20, 190-195.

Xuetong, F.; Thayer, D.W. Gamma-radiation influences on browning, antioxidant activity, and malondialdehyde level of apple juice. *J. Agric. Food Chem.*, **2002**, 50, 710-715.

Young, H.; Gilbert, J.M.; Murray, S.H.; Ball, R.D. Causal effects of aroma components on *Royal Gala* apple flavours. *J. Sci. Food Agric.*, **1996**, 71, 329-336.

Yulianti, F. *Flavor characteristics of irradiated apple cider* [MSc thesis]. Ames, IA: Iowa State Univ., **2003**; 119 pp. Available from: Iowa State University Parks Library, Ames, IA.

ACKNOWLEDGEMENTS

I feel honored to complete graduate study in the Dept. of Food Science and Human Nutrition at Iowa State University. I would especially like to thank my major professor, Dr. Terri Boylston, for the countless hours of help, patience and guidance she has dedicated to me throughout graduate school. I would also like to express gratitude to my committee members, Dr. Cheryll Reitmeier, Dr. Lester Wilson and Dr. Gail Nonnecke, for their advice and recommendations throughout this project. I would also like the thank Dr. Bonnie Glatz for the use of her microbiology laboratory, Dr. Philip Dixon for statistical advice, and department staff members who have been quite helpful and patient.

I have formed many friendships during my time at Iowa State University for which I am very grateful. I appreciate the constant help and support from many fellow graduate students in the food science department. I would especially like to thank Edwin Morris, Meg Stauder, Tonya Kouri and Bill Plebanski for their continuing friendship throughout my time at Iowa State University.

My family has also demonstrated encouragement throughout my education. I am very thankful to my parents for instilling a sense of pride and ambition in my work. My brothers and sisters have been an inspiration to complete any goals I wish for in my career. I am very grateful for all the blessings that have surrounded me with family and friends.